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UNIVERSITY OF ALBERTA

MS2 COLIPHAGE AND HPC BACTERIA AS INDICATORS OF OZONE DISINFECTION PERFORMANCE

BY

RONALD DAVID HELMER

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE

in

ENVIRONMENTAL ENGINEERING

Department of CIVIL ENGINEERING

Edmonton, Alberta

Fall, 1992

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled MS2 COLIPHAGE AND HPC BACTERIA AS INDICATORS OF OZONE DISINFECTION PERFORMANCE submitted by RONALD DAVID HELMER in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in ENVIRONMENTAL ENGINEERING.

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Dr. M. Belosevic

To my precious daughter Alina

ABSTRACT

A study into the effects of naturally varying water parameters on ozone disinfection of MS2 coliphage (ATCC 15597) and HPC bacteria was undertaken. A series of factorially designed experiments was conducted with control parameters of pH, turbidity, total hardness, temperature, DOC, and applied ozone dose. Most of the experiments were paired between the MS2 coliphage and HPC bacteria. All test runs were conducted with raw North Saskatchewan River water sampled during winter and during spring runoff. The experiments were, for the most part, conducted at bench scale using a Erlenmeyer reaction vessel and also at pilot scale using a semi-batch ozonation reactor. Some comparisons were made between the bench scale and pilot scale experiments. In addition, two trials of ozone inactivation of *Giardia muris* were done with the pilot scale reaction vessel. Several ozone demand experiments were also conducted to elicit the factors important to ozone demands.

MS2 coliphage was found to be exceedingly sensitive to ozonation. Up to 5 logs inactivation could easily be achieved. However, the presence of DOC material greatly decreased the ozone inactivation of MS2 coliphage by creating an ozone demand using all applied ozone before significant inactivation could occur. Higher temperatures may also increase the inactivation of MS2 coliphage. The other factors of pH and turbidity were not significant to ozone inactivation of MS2 coliphage. The HPC bacteria had only up to 3 logs inactivation for the higher ozone doses. The most significant factors affecting the inactivation of HPC bacteria were the ozone dose and the DOC. Similar to the MS2 coliphage, higher amounts of DOC retarded the inactivation of HPC bacteria. The paired inactivations between HPC and MS2 coliphage indicated that the MS2 coliphage had almost 3 logs higher inactivation than HPC bacteria for all sets of conditions. Pilot scale experiments also indicated that the MS2 coliphage is very sensitive to ozonation. Greater than 5 logs were consistently measured for any ozone residual. The pilot scale HPC inactivations were inconclusive since very little inactivation was observed. The ozone demand experiments suggested that the temperature and DOC concentration are important to the ozone demand.

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1.0 INTRODUCTION

1.1 INTRODUCTION

MS2 Coliphage and HPC bacteria were used as ozone disinfection indicators in raw surface water. The experiments were conducted at both bench and pilot scale with seeded MS2 coliphage and native HPC bacteria. The bench scale experiments investigated the ozone dose response of MS2 coliphage and also several factorial designed experiments investigating the effects of pH, turbidity, total hardness, temperature, DOC and applied ozone dose. The pilot scale experiments were conducted in a semi-batch ozonation reactor with seeded MS2 coliphage, seeded *Giardia muris* and native HPC bacteria. These experiments were ozone dose response experiments of these organisms to ozone in raw surface water.

This document is divided into eight general sections. The first section (Section 1) is the Introduction which describes the layout of this report in addition to the objectives of this study and the scope of work. Section 2 is the Literature Review of ozone chemistry, ozone disinfection, microbiology of water and a description of the current Canadian and US regulation concerning the microbiological quality of treated water. The materials and methods used in this study are described in Section 3. Materials covered are the water quality and characteristics, the bench and pilot scale reaction vessels, ozonation generators and measuring equipment, methods of analysis for both chemical and microbiological analysis and the statistical methods used to design the experimental developments and evaluate the results. The results of the experiments are presented in Section 4 for the bench scale disinfection, pilot scale disinfection, and ozone demand results. An investigation of the immediate ozone demand evolved from the results of the ozone disinfection experiments. The effects of DOC, temperature and filtering was investigated on the immediate ozone demand of raw surface water. The Discussion is covered in Section 5 discussing the results reported in Section 4 and in the Appendices. Section 6 summarizes and concludes the findings of this study and makes some recommendations which evolved from this study. All References used throughout this document are included in Section 7. The Appendices contain the detailed calculation of the statistical analysis and the bulk data summarized in the Results.

1.2 OBJECTIVES

The conditions which are best for ozone disinfection are those which maintain the highest ozone residual for longest time period. Several factors involved with the possible increasing of ozone decomposition are pH, DOC, alkalinity, and temperature. The pH, alkalinity and temperature are all implicated in ozone autodecomposition whereas DOC may act directly with ozone creating an ozone demand. Additionally, disinfection may be altered by microbial resistance changes due to turbidity. Microbes may be protected from disinfection by being adsorbed or imbedded in inorganic turbidity. Also organic turbidity may create an ozone demand similar to DOC.

The use of MS2 as a disinfection indicator evolved from its resistance to chlorine disinfection compared to other enteric viruses (Malcolm Pirnie Inc. and HDR Engineering Inc., 1991). However, other researchers (Evison, 1978; Finch and Fairbairn, 1991) indicated that MS2 coliphage may be too sensitive to ozone disinfection.

The objectives of this study include:

- Use MS2 Coliphage and HPC bacteria as indicators of disinfection performance.
- Compare the disinfection performance of MS2 coliphage and HPC bacteria with reported literature.
- Evaluate the use of MS2 coliphage as an indicator of ozone disinfection.
- Investigate the naturally varying parameters of pH, turbidity, total hardness, temperature, and DOC in raw North Saskatchewan River water.
- Conducts the experiments at both pilot and bench scales.

1.3 SCOPE OF WORK

This study involved a series of factorially designed experiments investigating the effects of ozone dose, pH, turbidity, total hardness, temperature, and DOC concentration of untreated North Saskatchewan River water on the inactivation of MS2 coliphage (ATCC 15597) and native HPC bacteria. The MS2 coliphage was used as a surrogate for enteric virus disinfection. The MS2 coliphage was cultured in the laboratory and seeded into the water samples prior to the experiments. The HPC bacteria were endemic to the river water. The water batches were subdivided into two general water types: winter (high quality) water and spring runoff (low quality water). The winter water was generally low in turbidity, dissolved organic carbon, temperature, and color. The spring runoff water had high turbidity, dissolved organic carbon, and visually noticeable color.

The relationships between the disinfection of water under varying conditions was studied. The first group of experiments were conducted varying the turbidity, pH, total hardness, and ozone dose. The second group of experiments were conducted varying the temperature, dissolved organic carbon, and ozone dose. Most of these experiments had paired MS2 coliphage and HPC bacteria. Pilot scale experiments were used to compare and evaluate the differences between pilot and bench scale experiment ozone inactivation of MS2 coliphage and HPC bacteria. With the bench scale experiments, the ozone was added as a sidestream, but the ozone was bubbled directly into the raw water for the pilot scale experiments. The results of the ozone disinfection experiments were compared with ozone disinfection of microorganisms of other studies.

Ozone demand experiments were also conducted on the raw surface water. Some of the water samples were filtered to remove the suspended organic carbon. The reason for filtering some of the samples was to experimentally determine if the ozone demand was predominantly from the suspended or dissolved fraction of the organic carbon. Other parameters tested in the ozone demand experiments include the effects of organic carbon concentration and temperature. These experiments were fashioned in a factorial design pattern and evaluated with one-way analysis of variance and with half normal plots.

2.0 LITERATURE REVIEW

2.1 OZONE CHEMISTRY IN WATER

2.1.1 General

Ozone is an allotropic form of oxygen which is produced by passing extra dry air or oxygen through a corona discharge. Ozone, an unstable gas, tends to revert back into molecular oxygen and must be generated on-site as required. The general reaction formula is shown below:

$$3O_2 + \text{energy} \rightarrow 2O_3$$

Ozone is a very powerful disinfectant and has one of the highest oxidation potentials known. Table 2.1 lists the oxidation potential for various chemical oxidants used in water and wastewater disinfection (Lange, 1961; CRC, 1991).

Table 2.1 - Oxidation Potentials for Various Chemical Disinfectants

Half Reaction	Standard Oxidation Potential, V
$\bullet OH + H^+ + e^- \leftrightarrow H_2O$	2.80
$F_2 + 2e^- \leftrightarrow 2F^-$	2.15
$O_3 + 2H^+ + 2e^- \leftrightarrow O_2 + H_2O$	2.08
$ClO_2 + 2H_2O + 5e^- \leftrightarrow Cl^- + 4OH^-$	1.91
$MnO_4^- + 8H^+ + 5e^- \leftrightarrow Mn^{2+} + 4H_2O$	1.51
$HOCl + H^+ + 2e^- \leftrightarrow Cl^- + H_2O$	1.48
$2H^+ + 2e^- \leftrightarrow H_2$	0.00

The most commonly found disinfectants are chlorine, chlorine dioxide and ozone. Ozone and chlorine dioxide are thought to act as an oxidant destroying cell walls and impairing cellular functions while free chlorine was believed to pass through the cell wall of bacteria and cause disruption of respiratory, transport and nucleic activity (Haas, 1990). Additionally, chlorine passes through viral capsid and reacts with the nucleic acids (Taylor, 1980). Kim *et al.* (1980) and Sproul *et al.* (1982) ozonated both DNA and RNA coliphages and determined that the method of virus inactivation was by ozone attack on the capsid and subsequent attack on nucleic acid.

2.1.2 Direct Reactions

Ozone is a chemical oxidant which will react with many organic and inorganic compounds. Some reactions with ozone are very reactive which quickly consumes all the residual ozone in solution. Additionally, residual ozone may also react by chain decomposition reactions which are discussed further in Section 2.1.3.

Direct reactions with ozone may include a variety of compounds found in surface, ground, or waste water. The ozone reaction with organics may be expressed by the first order rate equation presented below (Watt *et al.*, 1989; Hoigné and Bader, 1978):

$$O_3 + M \rightarrow M_{oxid}$$

Watt *et al.* (1989) studied the reaction kinetics of ozone in activated sludge. They indicated that the reaction rate constant is not "constant" in water but variable depending upon the kinetics of the organic compounds reacting with ozone. The equation below shows the first order rate equation with a pseudo first order rate constant, *w*, which can be described as the sum of all reacting species (Watt *et al.*, 1989; Staehelin and Hoigné, 1985; Gurol, 1985):

$$\frac{d[O_3]}{dt} = -w\,[M]\,[O_3]$$

The compounds with higher reaction rate constants would quickly and preferentially react with ozone in solution. The ozone would then subsequently react with the remaining compounds until completely consumed. Gurol (1985) also included the ozone decomposition rate constant with the specific ozone utilization coefficient, *w*. Thus, the specific ozone utilization rate can be expressed as:

$$w = \sum_i k_i[M_i] + k_d$$

Where k_i is the individual rate constants for the various solutes present and k_d is the autodecomposition rate of ozone. In high ozone demand situations (i.e. activated sludge or high DOC surface waters) the autodecomposition of ozone is considered small relative to the sum of the individual rate constants.

The specific ozone utilization rate may be explained in terms of pH, alkalinity and TOC. Yurteri and Gurol (1988) proposed an empirical relationship describing *w* for a variety of water conditions:

$$\text{Log } w = -3.98 + 0.66\text{pH} + 0.61 \log(\text{TOC}) - 0.42\log(\text{A}/10)$$

Where, w = specific ozone utilization rate, /hr
TOC = total organic carbon, valid between 1 and 5 mg/L
A = alkalinity, valid between 10 and 500 mg/L as $CaCO_3$
pH = valid between 7 and 9 pH units

Reactions of ozone with solutes also depend on the reaction time, ozone concentration, and solute concentrations. Some of the organic grouping which can be readily oxidized by ozone include olefinic and acetylenic carbon-carbon bonds; aromatic, carbocyclic and heterocyclic molecules; carbon-nitrogen and similar unsaturated groupings (Bailey, 1972).

Ozonation byproducts of direct reactions have been identified (Glaze *et al.*, 1989; Killops, 1986; Legube *et al.*, 1989) to include aldehydes, ketones, and carboxyclic acids. Aliphatic aldehydes, especially formaldehyde and heptanal, were major constituents of the ozonation disinfection by-products (Glaze *et al.*, 1989).

2.2.3 Chain Reactions

An important aspect of aqueous ozone chemistry is the autodecomposition of ozone in water. The two most important variables involved with ozone autodecomposition in "pure" water are temperature and pH (Stumm, 1958; Sullivan and Roth, 1980; Roth and Sullivan, 1983). Higher temperatures appeared to increase the rate of ozone decomposition by increasing the ozone reaction rate kinetics of ozone with free radical reacting species. Ozone will also decompose at higher reaction rates for increasing pH values by being reactive towards hydroxyl ions. The decomposition appeared independent of the pH for values less than pH 4.0. (Alder and Hill, 1950; Hewes and Davison, 1971; Gurol and Singer, 1982).

Many of the researchers had reported that ozone decay was either first, three-halves, or second order (or a combination of these) with respect to the ozone concentration (Teramoto *et al.*, 1981; Peleg, 1976; Gurol and Singer, 1982). Amid the varying reported decomposition orders of ozone, most researchers agreed that ozone is initiated by the hydroxyl ion and that the hydroxyl and hydroperoxy radicals are involved in the chain decomposition of ozone.

Chemicals involved in the chain reactions may be classified into one of three groupings: initiators, promoters, and terminators (Staehelin and Hoigné, 1985). Initiators are the chemicals (in either pure or natural water) that react directly with ozone and have by-products that, either directly or indirectly, continue to react with ozone. Promoters are chemicals which react with ozone reaction byproducts (i.e. hydroxyl radicals) and create other by-products which will also continue to react with ozone. Chain terminators react with the active ozone by-products (i.e. hydroxyl radicals) and form relatively inert products. Examples of promoters include formic acid, primary and secondary alcohols, and humics. Examples of scavengers include carbonate alkalinity, aliphatic alkyl compounds, and *tert* butyl alcohol. Some examples of initiators include hydroxyl ions, hydrogen peroxide, formate, humics and ferric ions.

After the initiation reaction, the main oxidizing intermediates formed in alkaline solutions are the hydroxyl and hydroperoxyl radicals (Taube, 1941; Staehelin and Hoigné, 1985). The hydroxyl radical is very reactive and non-selective. Hydroxyl radicals will react with inert products (Hoigné and Bader, 1978) which would probably be the nearest molecule. The two main models describing the autodecomposition of ozone are the Miami model (Tomiyasu *et al.*, 1985) and the HCW model (Staehelin and Hoigné, 1985). More is discussed on these models in the following two sections.

2.2.4 Miami Model

Tomiyasu *et al.* (1985) proposed that ozone decomposition may be described by a rate equation involving first and second order rate terms:

$$-\frac{d[O_3]}{dt} = k_1[O_3] + k_2[O_3]^2$$

The second order term is not observed when radical scavengers are present in solution. The initiation step is written below:

$$O_3 + OH^- \rightarrow HO_2^- + O_2$$

This initiation step is also confirmed by other researchers (Forni *et al.*, 1982) and corresponds to a two electron transfer process or an oxygen atom transfer from the ozone to the hydroxide ion.

The peroxide ion would react further with ozone to form ozonide and the superoxide free radical ions:

$$HO_2^- + OH^- + O_3 \rightarrow \cdot O_3^- + \cdot O_2^- + H_2O$$

The superoxide radical selectively reacts with ozone to produce more ozonide radical ions:

$$\cdot O_2^- + O_3 \rightarrow \cdot O_3^- + O_2$$

Moreover, the ozonide free radical ion decomposes into hydroxyl radicals which, in turn, react with ozone and produce additional superoxide free radical ions:

$$\cdot O_3^- + H_2O \rightarrow \cdot OH + OH^-$$

$$\cdot OH + O_3 \rightarrow \cdot O_2^- + H^+ + O_2$$

In brief, ozone is initiated by the hydroxide ion and reacts to form reactive products like the superoxide and hydroxyl free radical ions which, in turn, react and decompose more ozone. These reactions proceed until the ozone is completely decomposed and/or the reactive species form inert byproducts. The half life of ozone may be increased by adding compounds which react with the hydroxyl free radicals and form products that do not form into superoxide free radical ions.

The production of hydroxyl radicals was determined to be up to 0.55 ± 0.08 mol per mol of ozone (Hoigné and Bader, 1976). Scavenging these hydroxyl radicals would increase the half life of ozone in water by removing the most reactive species in water which continues the chain reaction with ozone. Conversely, certain chemicals will enhance hydroxyl radical formation and the half life of ozone will be considerably shorter (Ferguson, 1990).

Figure 2.1 illustrates the full reaction scheme for the autodecomposition of ozone in "pure" water.

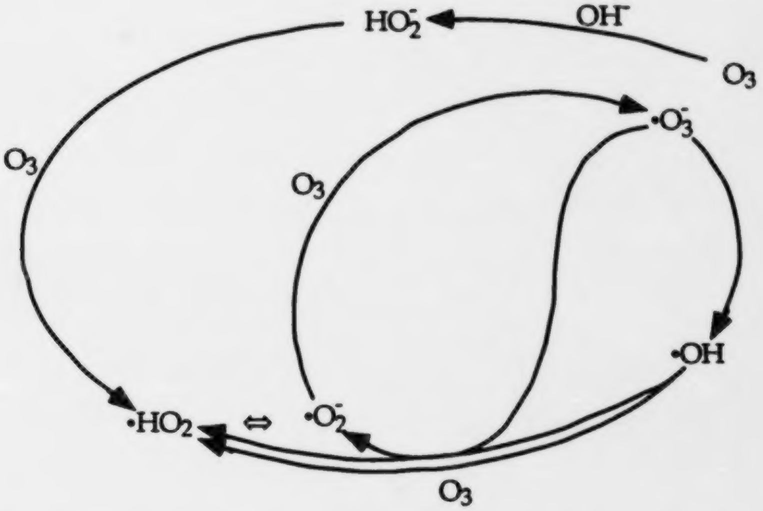


Figure 2.1 - Miami Reaction Scheme for the Autodecomposition of Ozone in Pure Water

2.2.5 HCW Model

Hoigné and co-workers suggested (Staehelin and Hoigné, 1985) an ozone decomposition reaction model similar to the one first proposed by Weiss (1935).

Ozone, in water, may act directly with dissolved substances and form ozone inert products or it may decompose to form secondary oxidants such as superoxide and hydroxyl ion free radicals. The reaction of hydroxyl ions with ozone is the initiation step which leads to the formation of superoxide ion and hydroperoxyl free radicals. The superoxide radical will protonize and decompose into the hydroxyl radicals (see Figure 2.2 and the equations below):

$$O_3 + OH^- \rightarrow \cdot HO_2 + \cdot O_2^-$$

$$O_3 + \cdot O_2^- \rightarrow \cdot O_3^- + O_2$$

$$H^+ + \cdot O_3^- \rightarrow \cdot OH + O_2$$

$$\cdot OH + O_3 \rightarrow \cdot HO_2 + O_2$$

Similar to the Miami model, the superoxide free radical ion and hydroxyl free radical tend to continue the decomposition of ozone through chain reactions. Termination reactions would form by interrupting the reaction sequence by reacting with the hydroxyl radicals and forming secondary products which do not form into the hydroperoxyl or superoxide ion free radicals.

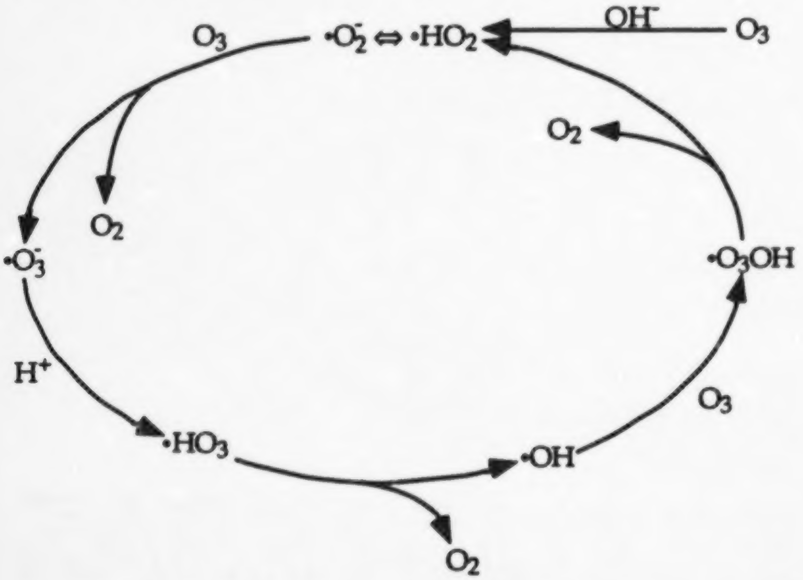


Figure 2.2 - HCW Reaction Scheme for the Autodecomposition of Ozone in Pure Water

Natural waters contain many chemicals which act as promoters, initiators, and terminators. In these reaction pathways, the ozone may react directly with a substance and produce an ozonide radical ion by electron transfer (Staehelin and Hoigné, 1985) or produce relatively inert products (see Figure 2.3). The hydroxyl radicals do not react with the ozone molecules since the reaction of hydroxyl radicals is such that it will immediately react with almost any other nearby species. However, some organic free radicals undergo auto-oxidation and the electron transfers to the oxygen and a superoxide ion free radical results. The superoxide free radical ions are selective towards ozone and will react with ozone to continue chain decomposition (Hewes and Davison, 1971; Taube, 1941; Staehelin and Hoigné, 1985).

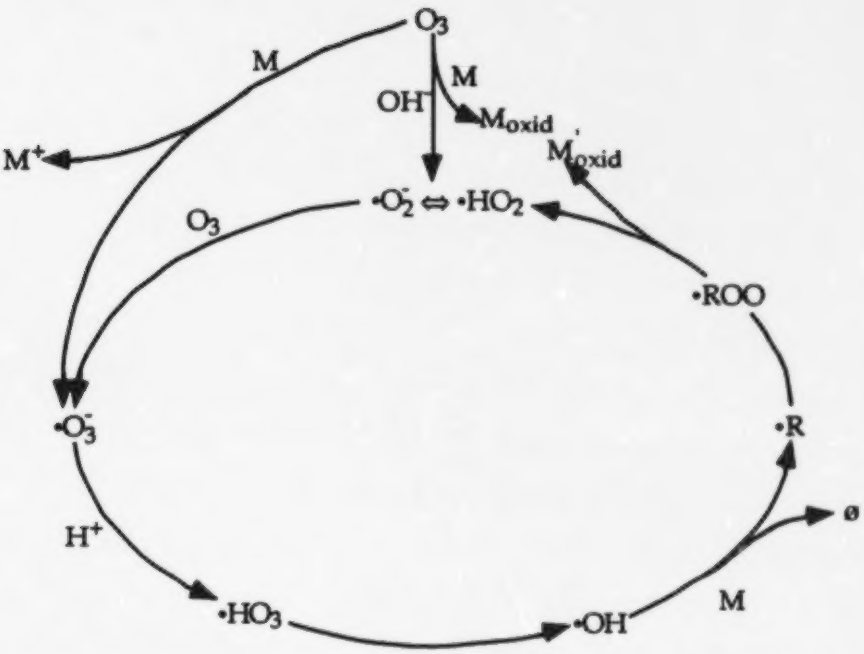


Figure 2.3 - HCW Reaction Scheme for the Autodecomposition of Ozone in Natural Water

The main differences between the Miami and the HCW mechanisms is that the HCW mechanisms is characterized by an oxygen radical transfer from ozone to the hydroxide ion whereas the Miami model corresponds to an oxygen atom transfer between ozone and the hydroxyl ion (Chelkowska *et al.*, 1990).

2.2 EFFECT OF WATER QUALITY PARAMETERS ON OZONE DISINFECTION

Certain water quality parameters may influence the effect of ozone disinfection by either creating an ozone demand, decomposing ozone at a faster rate, or physically protecting the microbes from ozonation. The water quality parameters discussed below in the following sections include pH, turbidity, alkalinity, temperature, and organics.

2.2.1 pH

The pH is important to the disinfection of water by both chlorine and ozone. The importance to ozone, as was indicated in Section 2.2.3, was that ozone decomposes at a faster rate in high pH waters. The results of the decomposition are superoxide ion and hydroxyl free radicals. While the superoxide ion free radicals are very selective towards ozone and would further decompose ozone directly, the hydroxyl radicals would likely react with the first available compound and create other reaction byproducts (which may further decompose the ozone by eventually producing superoxide free radical ions). It is possible that some of these hydroxyl radical will react and inactivate some of the microorganisms, however, it is more likely that the hydroxyl radicals will react with the nearest molecule before inactivating any microorganism (Hoigné and Bader, 1975). The decomposition of ozone into hydroxyl radicals may be detrimental to the inactivation of microorganisms.

Farooq *et al.* (1977b) indicated that pH has only minimal effect on ozone disinfection of yeast (*Candida parapsilosis*) and acid fast bacteria (*Mycobacterium fortuitum*) and that the autodecomposition (due to pH) was not important in the inactivation. This suggests that the ozone reaction rate with the organisms was almost immediate in the phosphate buffer solution used in the experiments. Similarly, Vaughn *et al.* (1987; 1990) determined that the pH was not related to the inactivation of hepatitis A virus or rotavirus in phosphate-carbonate buffers between the pH of 6 and 8. Here again, the decomposition rate was seemingly not as important as the presence of an ozone residual. Roy *et al.* (1982) determined that viral inactivation was enhanced by a change in pH. At lower pH the inactivation of poliovirus 1 and echovirus 1 was lower than at higher pH in tripled distilled water. At higher pH values of about 7.0 made the viruses more sensitive to ozonation than the lower pH values of about 4.3. Smith and Bodkin (1944) in early experiments had also concluded that ozone did not effect the inactivation of bacteria over a pH range of 5 to 9. Hoff (1986) and Hoff and Akin (1986) also indicated that there is no direct pH effect on inactivation of bacteria by ozone.

2.2.2 Turbidity

Turbidity may be in the form of organic turbidity consisting of cellular matter, decomposition matter, or feces and turbidity may be in the form of inorganic clays and silts. Geldreich (1983) reported that approximately 60 percent of the biomass and HPC activity in river water is associated with suspended solids. Hoff (1978) determined that the particles associated with inorganic turbidity are offered no protection from ozonation, however, viruses and bacteria associated with cell debris, feces or wastewater effluent solids are substantially protected from ozonation. Other researchers investigating the effect of suspended solids on the inactivation of ozone on viruses and bacteria have indicated similar findings. Foster *et al.* (1980) indicated that protection of cell and fecal associated viruses and bacteria may occur by competitive oxidation. Coliforms and poliovirus 1 were protected by organic matter from ozonation. Additionally, it was determined (Emerson *et al.*, 1982) that picornaviruses were protected by being cell associated. It appears, however, that not all viruses may react the same to clay and ozonation. The viruses poliovirus 1, coxsackievirus A9, and *E. coli* bacteria were associated with bentonite and were not protected from ozonation. However, bentonite clay retarded the ozone inactivation of f2 coliphage (Boyce *et al.*, 1981). Note that the f2 coliphage does not have the same adsorption characteristics as the poliovirus 1 (Moore *et al.*, 1975) which may have some influence on the relatively different inactivation characteristics of these two viruses. Walsh *et al.* (1980) determined that the aluminum hydroxide floc resulting from coagulation did not impede the inactivation of either bacteria or viruses. These metal hydroxide flocs were not reactive to ozonation.

Viruses will readily adsorb onto inorganic clays (Schaub *et al.*, 1974). Divalent cations and low pH values both have a net effect of increasing the viral adsorption to clay particles. The presence of microbial overgrowth and organic coatings decreases the virus adsorption to the inorganic clays (Fuhs *et al.*, 1985). Two theories of viral adsorption suggested (Schaub *et al.*, 1974) are briefly summarized below:

- First, viruses and clays generally have a net negative charge. By lowering the pH or adding a cation, the solution is destabilized and viral adsorption to clay particles increases.

- Secondly, it is possible that cations can provide a bridge between the viruses and clays. Having more cations would allow greater numbers of viruses to adsorb to the clay particles. Resolution of the cations would cause elution of the viruses.

In any event, the viruses adsorbed onto the clay particles still retain their infectivity (Moore *et al.*, 1975; Schaub and Sagik, 1975). This has serious implications to the treatment and disposal of sludges from water and wastewater treatment plants.

2.2.3 Alkalinity

Carbonate alkalinity may be important to the inactivation of microbes by scavenging the hydroxyl radicals and maintaining a more stable ozone solution. Doré *et al.* (1987) showed that the ozone residual lasted longer in solution when bicarbonate ions were present. Furthermore, they demonstrated that a greater amount of fulvic acid reacted when bicarbonate was also present in the water. The bicarbonate (or carbonate) would terminate the chain reactions by scavenging the hydroxyl radicals according to the reaction mechanism below (Adams *et al.*, 1965):

$$CO_3^{2-} + \bullet OH \rightarrow \bullet CO_3^{-} + OH^{-}$$

Ozone disinfection of *E. coli* was studied by Finch *et al.* (1992) in phosphate and carbonate-phosphate buffers. They concluded that alkalinity significantly inhibited ozone disinfection of *E. coli* in ozone demand free water and that direct reactions were responsible for the inactivation of the bacteria.

2.2.4 Temperature

Temperature may be important to the inactivation of viruses and bacteria by either increasing the decomposition rate of ozone or by altering the virus resistance to ozonation. Wickramanayake and Sproul (1988) inactivated poliovirus 1 and amoeba cysts with ozone over the temperature range of 5 to 30°C. They found that the inactivation increased with the temperature. *Mycobacterium fortuitum* inactivation by ozone was also increased at higher temperatures for temperatures of 9 to 37°C (Farooq *et al.*, 1977b). A lower temperature of 10°C had resulted in a higher inactivation rate for hepatitis A virus and *E. coli* than at 20°C (Herbold *et al.*, 1989). However, under the same experimental conditions, there was no apparent increase in inactivation rates for poliovirus 1. Virus survival is effected by temperature alone without any applied chemical oxidants. It was determined that temperature is a major factor in the "natural" inactivation of viruses (Kutz and Gerba, 1988). As the temperature increases the natural die off of viruses significantly increases.

Lowering the temperature from 5 to 1°C had little effect on the inactivation kinetics of poliovirus 1, coliphage T2, and *E. coli* in distilled ozone demand free water (Katzenelson *et al.*, 1974). The temperature of the surrounding medium or water was observed to affect viral inactivation by ozone (Roy *et al.*, 1982). An increase in temperature resulted in a decrease in resistance to inactivation by ozone in ozone demand free phosphate buffer.

2.2.5 Organics

Organics dissolved in water play an important role with ozone disinfection by creating an ozone demand. These organics can react with a considerable portion of the applied ozone leaving less available for ozone oxidation and disinfection.

Humic materials constitute up to 50% of the DOC in water (Thurman and Malcolm, 1981). Humic substances complex metals, act as buffers, solubilize organic compounds, and generate THMs during chlorination. Additionally, humic substances add color to the water and react with ozone (Anderson *et al.*, 1986; Christman and Ghassemi, 1966).

The size variation of humic substances is considerable ranging from 500 to 10,000 g/mol. Fulvic acids have smaller molecular weights ranging between 500 to 2000 g/mol. Humic acids have higher molecular weights ranging from 1000 and 10,000 g/mol. The average molecular weight of humic substances in the water was reported to be approximately 500 g/mol (Anderson *et al.*, 1986; Christman and Ghassemi, 1966). Moreover, it was determined that fulvic acids are the predominant fraction of humic substances in water (Trussell and Umphres, 1978).

Color is usually associated with the humic materials. Humic substances causing color have terrestrial origin which may arise from (Christman and Ghassemi, 1966) any of the following:

- Aqueous extraction of living woody substances,
- Solution of degradation products in decaying wood,

- Solution of soil organic matter, or
- Combinations of the above.

Shariro (as reported in Christman and Ghassemi, 1966) suggested that humics causing color are predominantly aliphatic in nature, however, Black and Christman (1963) indicated that color is caused by aromatics. This was also confirmed (Amy *et al.*, 1986) that humic and fulvic acids have an aromatic infrastructure. Figures 2.4 and 2.5 are suggested chemical structures of fulvic and humic acids, respectively (after Christman and Ghassemi, 1966). Note that both of these compounds contain a large number of aromatic rings which may be reactive to ozonation and potentially cause a relatively high ozone demand.

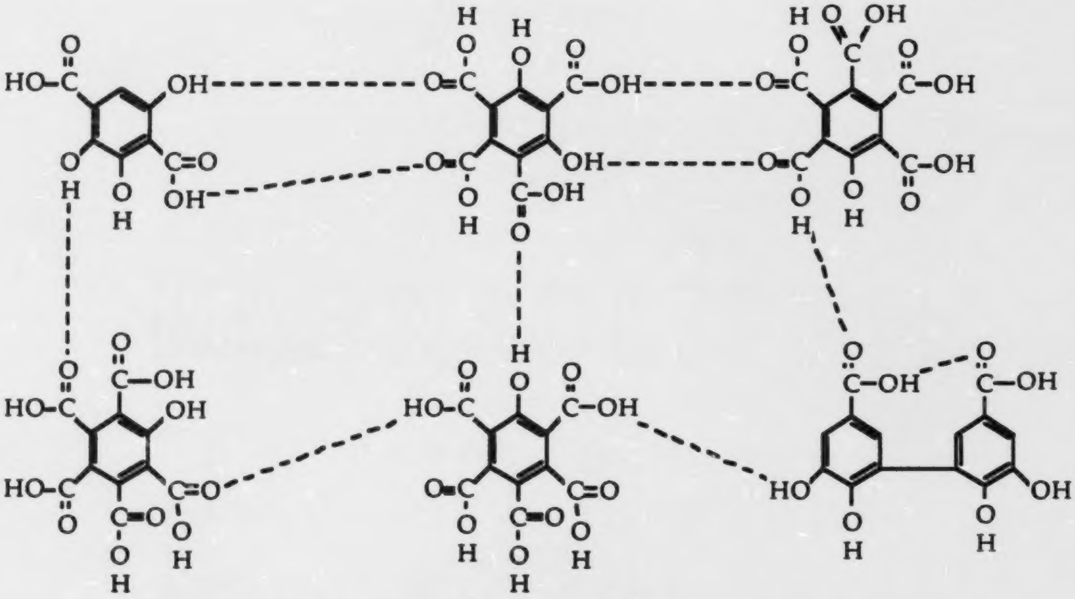


Figure 2.4 - Suggested Structure of Aquatic Fulvic Acid

One of the striking properties of ozonation of fulvic and humic acids is that the color immediately disappears as soon as the ozone is added and that the UV absorbance decreases (Black and Christman, 1963; Anderson *et al.*, 1986; Killops, 1986; Fløgstad and Ødegaard, 1985; Amy *et al.*, 1988; Kruithof *et al.*, 1989). It was determined that ozone reacted first order with humics (Mallevaille, 1975). The reaction occurs first at the sensitive nucleophilic sites and, at the higher dosages, cleavage of the macromolecules (Anderson *et al.*, 1986). Generally, there was a shift to lower molecular weight compounds but not a considerable decrease in DOC (Legube *et al.*, 1989; Anderson *et al.*, 1986; Fløgstad and Ødegaard, 1985; Amy *et al.*, 1988; Kruithof *et al.*, 1989) or production of carbon dioxide (Maier, 1982).

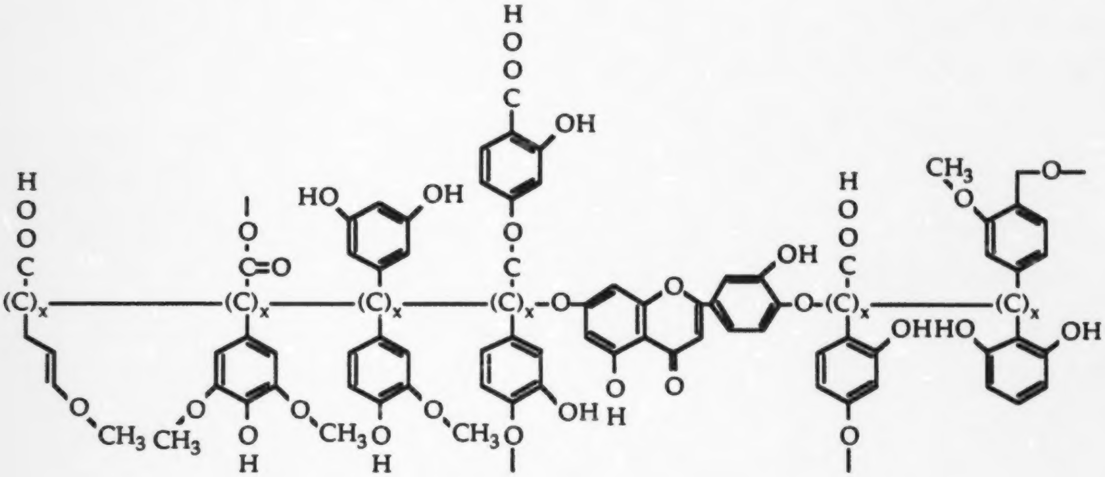


Figure 2.5 - Suggested Chemical Structure of Aquatic Humic Acid

2.3 MICROBIOLOGY OF WATER

Microorganisms in water may constitute either viruses, bacteria, protozoa, or helminths. Each of these may have various pathogenic species which may be transmitted in drinking and recreational water. Other concerns regarding microorganisms and drinking water are the opportunistic pathogens and the organisms which regrow in water distribution systems. The opportunistic pathogen may cause disease or infection when a host's defenses are stressed or impaired. Regrowth of bacteria in drinking water causes build-up of biofilms and may result in higher chlorine residual demand, taste, odor, and color. Additionally, iron reducing bacteria may enhance corrosion of iron pipes resulting in premature pipe failure.

Craun (1988) reported the incidence of waterborne disease outbreaks between 1971 to 1985 for the United States (see Table 2.2). Most of the waterborne disease outbreaks are from unknown origin which is not surprising since an outbreak may be short lived and it may sometimes be difficult to identify the agent(s) responsible for the outbreak.

Table 2.2 - Waterborne Disease Outbreaks in the United States (1971 - 1985)

Illness	Number of Outbreaks	Cases of Illness
Unidentified Gastroenteritis	251	61,478
Giardiasis	92	24,365
Shigellosis	33	5,783
Hepatitis A	23	737
Viral Gastroenteritis	20	6,254
Campylobacterosis	11	4,983
Salmonellosis	10	2,300
Typhoid	5	282
Yersiniosis	2	103
Toxic *E. coli* Gastroenteritis	1	1,000
Cryptosporidiosis	1	117
Cholera	1	17
Dermatitis	1	31
Amebiasis	1	4
Total	452	107,454

(after Craun, 1988)

Giardiasis account for the most outbreaks and most numbers of illnesses caused. Many of the remaining outbreaks were caused by more "traditional" waterborne diseases. Figure 2.6 relates the disease outbreaks listed in Table 2.2 with the type of water treatment plants and/or associated deficiencies.

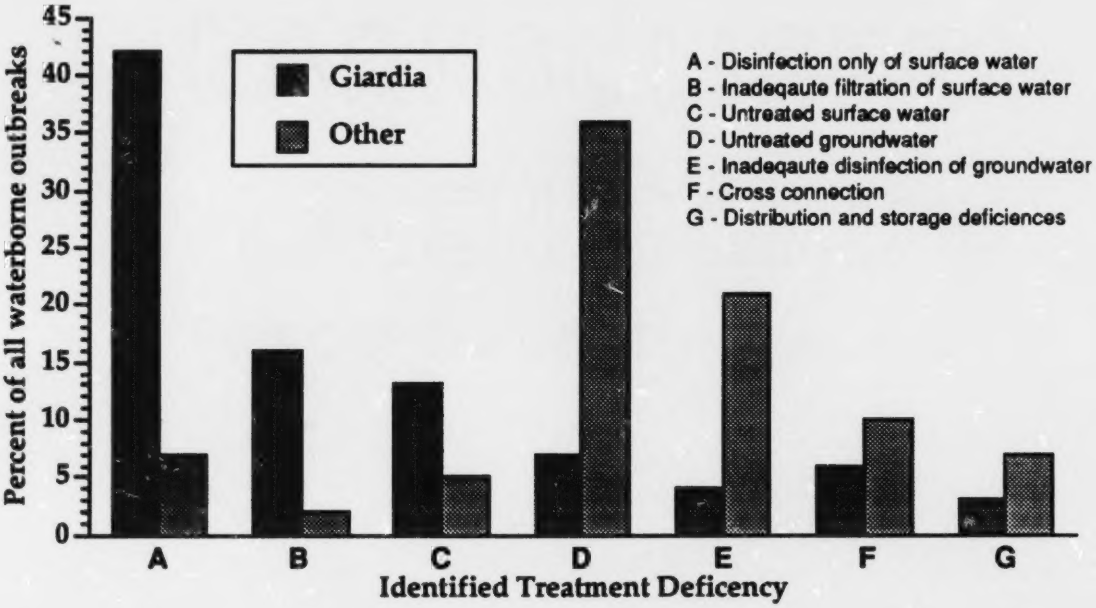


Figure 2.6 - Waterborne Disease Outbreaks Associated with Various Types of Water Treatment (after Craun, 1988)

Most of the disease outbreaks listed above occurred in water supplies where the water was treated by disinfection only or in untreated groundwater supplies. Disinfection (alone) of a surface water supply allows large numbers of *Giardia* cysts into drinking water supplies. Additionally, groundwater is not always protected from viral contamination. It appears that many communities consider groundwater free from microbial pollution and only have only minimal or no disinfection. These types of outbreaks prompted the USEPA to regulate the disinfection of all ground and surface waters to a minimum standard.

Canadian statistics of reported (Health and Welfare Canada, 1991, 1989b, 1987, 1986) diseases are listed in Tables 2.3 and 2.4 for Canada and Alberta, respectively. Note that the diseases listed are also associated with waterborne disease outbreaks, however, no distinction is made on the transmission route of these diseases. Caution must be exercised when interpellating these statistics since many of these disease occurrences, if not all, have multiple vectors of transmission. Possible transmission routes of these diseases include (Smith, 1968):

- Direct person to person (*Salmonella*, etc.)
- Infected Food (*Salmonella, Shigella*, etc.)
- Infected Animals (helminths, etc.)
- Waterborne Diseases (*Cholera, Giardia*, etc.)

Table 2.3 - Notable Disease Outbreaks in Canada (1984 to 1990)

Disease	1990	1989	1988	1987	1986	1985	1984
Amoebiasis	1863	2265	1792	1841	1548	1622	1477
Campylobacteriosis	9234	11,380	9332	10,068	9631	N/A	N/A
Cholera	-	-	1	-	1	-	-
Giardiasis	7868	9269	8224	8665	8233	7090	6599
Hepatitis A Virus	1584	1788	1369	976	1321	2462	2400
Legionellosis	67	69	39	48	66	N/A	N/A
Paratyphoid	14	26	18	28	31	16	24
Poliomyelitis	-	1	1	-	-	-	1
Salmonellosis	7088	10,432	9629	10,884	9955	7481	10,869
Shigellosis	1148	1845	1596	1315	1415	1231	1977
Typhoid	63	81	52	36	50	41	64

Table 2.4 - Notable Disease Outbreaks in Alberta (1984 to 1990)

Disease	1990	1989	1988	1987	1986	1985	1984
Amoebiasis	133	134	125	92	117	111	109
Campylobacteriosis	873	982	799	714	857	N/A	N/A
Cholera	-	-	-	-	-	-	-
Giardiasis	1360	1475	1357	1279	1555	1489	1340
Hepatitis A Virus	272	274	193	109	312	462	214
Legionellosis	10	4	6	12	7	N/A	N/A
Paratyphoid	1	1	3	11	10	1	3
Poliomyelitis	-	-	-	-	-	-	-
Salmonellosis	806	1058	876	781	714	732	726
Shigellosis	110	160	237	170	140	195	232
Typhoid	5	6	7	2	3	5	12

2.3.1 Common Pathogens

Viruses

There are over 100 enteric viruses found in the human feces. All of these viruses are presumed to be waterborne and cause disease. All enteric viruses come from the picornavirus family with the exception of hepatitis A virus which, as yet, has not classified (Kucera, 1983).

Enteric viruses are those which constitute their existence by a fecal-oral route. These viruses include adenoviruses, reoviruses, rotaviruses, enteroviruses, hepatitis A virus, and the Norwalk and related gastrointestinal group. Table 2.5 shows enteric viruses which may be present in water.

Table 2.5 - Viruses Present in Water

Virus Group	Number of Types	Disease Caused
Enteroviruses		
Poliovirus	3	Paralysis, meningitis, fever
Echovirus	34	Meningitis, repiratory disease, rash, diarrhea, fever
Coxsackievirus A	24	Herpangina, respiratory diseases, meningitis, fever
Coxsackievirus B	6	Mycocarditis, congenital heart anomalies, rash, fever, meningitis, respiratory disease
Enterovirus types 68 - 71	4	Meningitis, encephalitis, respiratory disease, acute hemorragic conjuncivitis, fever
Hepatitis type A	1	Infectious hepatitis
Norwalk type Virus	2	Epidemic vomiting and diarrhea fever
Rotavirus	2	Vomiting and diarrhea fever
Reovirus	3	Not clearly established
Adenovirus	>30	Respiratory disease, eye infections

(after Melnick and Gerba, 1982)

Viruses are strictly obligate parasites since they require a host cell to multiply. They will not propagate outside of their natural host but may be found in ground, tap, river and impounded water. Survival of viruses in these environments appears to decrease slowly with time and also depends on the type of water and temperature (Kutz and Gerba, 1988).

Viruses are very small and extremely simple organisms. Most viruses are less than 100 nm in diameter but their sizes range from 20 to 300 nm. This small size makes them exceedingly difficult to remove in filters in water treatment plants. The basic virus consist of a core of DNA or RNA nucleic acid surrounded by a protein capsid (Montgomery, 1985). Some have protective lipid envelopes which allows the virus to escape antibodies detection by altering their lipid envelope (i.e. cold or flu viruses).

Bacteria

Bacteria are single-celled organisms ranging in size from 0.1 to 10 µm with a simple procaryotic structure. Bacteria may be classified as either autochthonous or allocthonous referring to bacteria which appear to naturally belong (autochthonous) while the allocthonous group refers to bacteria which came from contamination, runoff, rainfall, and so on. This later group generally have a limited life in the water. Of specific concern are the bacteria which arise from fecal contamination because of the possibility of transmission of waterborne disease.

Bacteria of public health significance in water are shown in Table 2.6. These bacteria are enteric bacteria and enter water sources by direct human or animal contact or indirectly by sewage input, urban or rural runoff. Microbial contamination is usual lowest in groundwater and highest in rivers but depends entirely upon human and animal activities (Harf and Monteil, 1989).

Table 2.6 - Bacteria of Health Significance in Water

Genus	Species	Host(s)
Salmonella	Several hundred serotypes known pathogenic to man. Examples include: *S. typhi*, *S. enteritides*, *S. typhimurium*	Typhoid fever (from *S. typhi*), salmonellosis from other species both causing acute diarrhea and cramping, typhoid fever may be fatal
Shigella	Four species cause disease in man: *S. sonnei*, *S. flexneri*, S. *boydii*, *S. dysenteriae*	Shigellosis, or bacillary dysentery, causes fever and bloody diarrhea
Leptospira	Common human isolates include *L. pomona*, *L. autumalis*, *L. australis*	Leptospirosis: acute infections of kidney, liver, central nervous system
Pasturelia	*P. tularensis*	Tularemia: causes chills and fever, with an ulcer at the site of infection, swollen lymph nodes
Vibrio	*V. Cholera*	Cholera: an acute intestinal disease causing vomiting, diarrhea, dehydration, may be fatal
Enteropathogenic *Escherichia coli*	Various serotypes	Diarrhea, urinary infections
Yersinia	*Y. enterocolitica*, *Y. pseudotuberculosis*	Diarrhea, fever, vomiting, anorexia, acute abdominal pain, abscesses, septicemia
Mycobacterium	*M. tuberculosis*, *M. balnei*, *M. bovis*	Pulmonary or skin tuberculosis
Camplylobacter	C. *fetus* subspecies *jejuni*	Campylobacterosis: sudden onset of abdominal pain, fever, headache and watery diarrhea. Blood may be present in diarrhea.
Legionella	*L. bozemanii*, *L. micdadei*, *L. dumoffii*, *L. longbeachae*, *L. pneumophila*	Pontiac fever: self-limiting non-pnuemonic form of legionellosis characterized by high infectivity, short incubation, and relatively mild illness. Also causes the disease legionellosis which is a possibly fatal (15 to 20% of cases) characterized by fever, malaise, chills, moderate cough, myalgia and headache.

(after Dufour, 1983; Brown, 1983; McKinney and Thomason, 1983; Montgomery, 1985)

Organisms most encountered in clinical cases are the enteric bacteria which refer to the bacteria which enter into the body orally. The normal habitat of these organisms is the intestinal tract. Enteric bacteria covers the bacteria of the families Enterobacteriaceae and Vibrionaceae. Almost any of the

Enterobacteriaceae and Vibrionaceae can produce disease in humans. These enteric organisms are not normally known as vigorous invaders of normal human tissue but, for the most part, are opportunistic human pathogens with the exceptions of *Shigella*, certain *Escherichia*, *Salmonella*, and *Vibrio* genera (Domingue, 1983).

Legionella appears to exist in a variety of environments ranging from freshwater lakes, rivers, wet solids, sewage, hot water tanks, potable water and so on. The *Legionella* are predominantly water associated. Legionellosis is usually contracted from the respiratory route but the bacteria are harbored in water sources (McKinney and Thomason, 1983). Muraca *et al.* (1988) reported that *Legionella pneumophila* was implicated in water transmission of this disease. Some of the factors controlling colonization of the water distribution include water temperature, sediment, scale, and presence of other bacteria.

Protozoa

Protozoa are eucaryotic, unicellular animals that are found either free living in the environment or as parasites infecting humans, plants, or animals. Some protozoa which may infect animals may also infect man (Daly, 1983). Some important protozoan pathogens include *Giardia lamblia*, *Entamoeba histolytica*, and *Cryptosporidium*. These microorganisms may infect the host by fecal-oral route and have been implicated in waterborne disease outbreaks.

Some protozoans lead a two stage life cycle comprised of a trophozoite stage and the cyst stage. Trophozoites are active and feeding forms of the protozoan. When protozoans are subjected to adverse conditions, they become inactive and surround themselves with a resistant membrane. It is because the cysts are so resistant to inactivation, that they are responsible for the spread of protozoan infections (Smith, 1968).

Table 2.7 lists the protozoa implicated in waterborne disease outbreaks.

Table 2.7 - Potential Waterborne Protozoan Pathogens

Protozoan	Host(s)	Disease	Transmission
Acanthamoeba castellani	Fresh water, sewage, humans	Amebic meningoencephalitis	Gains entry through abrasions, ulcers, and as secondary invader during other infections
Balantidium coli	Pigs, humans	Balantidiasis (dysentery)	Contaminated water by pig feces
Entamoeba histolytica	Humans	Amoebic dysentery	Contaminated water
Giardia lamblia	Animals, humans	Giardiasis (gastroenteritis)	Contaminated water
Nagleria fowleri	Soil, water, decaying vegetation	Primary amoebic meningoencephalitis	Nasal inhalation with subsequent penetration of nasopharynx; exposure from swimming in freshwater lakes
Cryptosporidium	Animals, humans	Cryptosporidiosis	Contaminated water

(after Montgomery, 1985; Rose, 1990)

The three most important protozoans of waterborne health significance are *Giardia lamblia*, *Cryptosporidium*, and *Entamoeba histolytica* which are able to survive in the environment as cysts. In the cyst form, protozoans are protected from adverse environmental conditions such as pH, light, temperature and oxidants. Of these three potentially pathogenic protozoans, *G. lamblia* and *Cryptosporidium* are on the increase as identified causes of waterborne disease outbreaks. The other three protozoans listed in Table 2.7 are not commonly known for waterborne disease outbreaks (Montgomery, 1985).

Cryptosporidium may be transmitted by water, however, it is not certain how frequent this protozoa will cause problems in water treatment systems. Rose (1988) suggested that the risk of *Cryptosporidium* transmission by the water route may be equal or greater than *Giardia*.

Cryptosporidium was reported (Rose *et al.*, 1988) to be positively correlated with *G. lamblia* in surface waters. This suggests that waterborne outbreaks of *Giardia* may be possibly linked to unrecognized waterborne outbreaks of *Cryptosporidium*. Furthermore, it is uncertain how effective filtering surface waters will be in considering the relatively small size of the oocysts and resistance to disinfectants (Rose, 1990).

2.3.2 Indicator Organisms

Disease causing pathogens are often difficult or impossible to detect before an outbreak without prior knowledge of their presence. Additionally, pathogenic organisms require special facilities to culture and enumerate. Indicator organisms should possess the following characteristics in order to be effective (Dadswell, 1990; Montgomery, 1985):

- abundant in feces and sewage
- absent or at least in very small numbers from all other sources
- capable of easy isolation, identification, and enumeration
- unable to grow in the natural aquatic environment
- more resistant than pathogens to disinfectants and environmental stress
- numbers should correlate with degree of pollution
- present in greater numbers than pathogens
- should survive equal to or greater than pathogens
- harmless to humans

No group of organisms currently meets all of these criteria (Montgomery, 1985); but the coliform bacteria group has long been used as an indicator of fecal pollution and disinfection. Microbes that have been used as various indicator organisms include (IAWPRC, 1991):

- indicators of fecal pollution (*E. coli*)
- sewage pollution (*Pseudomonas aeraginosa*)
- differentiate between human and animal fecal pollution (*fecal coliform to fecal streptococci ratio*)
- age of fecal pollution (*spores of Clostridia*)
- indicators of nutrient pollution (*Aeromonas hydrophila*)
- disinfection indicators (*E. coli, Mycobacterium fortuitum*, coliphage MS2, coliphage f2, etc.)

Coliforms

Standard Methods (APHA, 1989) indicated that coliform bacteria comprise all the aerobic and facultative, gram negative, non-spore forming, rod-shaped bacteria which ferment lactose with gas formation within 48 hours at 35°C.

One of the main reasons for coliform bacteria used as an indicator is that approximately 1.95 billion bacteria are excreted by each person per day. This would give an indication of sewage or feces contamination (Krenkel and Novotny, 1980). Coliform bacteria will not, under most circumstances, reproduce in temperatures below 30°C (Havelaar and Pot-Hogeboom, 1988). Therefore, coliform bacteria can only proliferate in the intestines of warm blooded animals.

Fecal coliform bacteria have similar growth and survival as the pathogens *Salmonella* and *Shigella* (Krenkel and Novotny, 1980). This also suggests that the presence of fecal coliforms could indicate the possible presence of enteric bacteria in water.

Fecal Strepptococci

It has been shown (Geldreich *et al.*, 1968) that waters which have a higher fecal coliform count than fecal streptococci are more likely to contain wastes of human origin. If the fc/fs (fecal coliform:fecal streptococci) ratio is greater than 4, it is nearly certain that the pollution is of human origin, while if the fc/fs is less than 0.7 it is nearly certain to be of animal origin. Intermediate values are less dependable, however. Any fc/fs ratios greater than 2 are likely to be human origin while fc/fs ratios less than 1 are likely to be animal sources. Intermediate values have little certainty of the origin (Steel and McGhee, 1979).

Heterotrophic Plate Count (HPC) Bacteria

Heterotrophic bacteria are a group of bacteria referring to the aerobic, facultative, or anaerobic bacteria which utilize organic carbon for energy and cell synthesis (Clark *et al.*, 1971).

The heterotrophic plate count is a Standard Method (APHA, 1989) which provides an estimate of the aerobic and facultative heterotrophic bacteria present in water. Some of the common HPC organisms include:

- *Aeromonas*
- *Alcaligenes*
- *Aeromonas listeria*

- *Flavobacterium mycobacterium*
- *Pseudomonas*
- *Plesiomonas*

Some of these organisms may be able to grow in water distribution systems. Factors affecting their growth include temperature (higher temperatures give greater potential for growth), water velocity and residence time (Rose, 1990). LeChevallier (1990) reported that HPC regrowth occurs in water distribution systems which are unable to maintain a residual of 0.2 mg/L or more of chloramines. These conditions resulted in HPC counts greater than 500 CFU/mL.

The HPC bacteria in rivers were found to change with environmental factors such as seasonal temperature change and rainfall events. Bell *et al.* (1982) determined that organic carbon, inorganic carbon and rainfall were strongly correlated with the HPC diversity and counts. Apparently many of the HPC bacteria were from terrestrial origins and were washed into the river with the surface runoff of the rain.

Seasonal fluctuations have pronounced effects on HPC bacteria numbers. The HPC numbers appeared to increase with temperature. However, HPC bacteria also increased downstream of rivers where human activity also increased (Nuttall, 1982a; 1982b). Trentham and James (1981) also indicated that the heterotrophic activity of the river changed seasonally. The types of bacteria dominant during the winter time were not dominant during the summer time and visa versa. Throughout the year long study, there was a discernable shift in bacteria types. During the winter time, the bacteria were mainly psychrophilic while during the summer time the bacteria were mainly mesophilic.

Mycobacterium

Mycobacterium has been suggested as a disinfection indicator of viruses and bacteria (Engelbrecht *et al.*, 1979). The relative resistance of the *Mycobacterium fortuitum* to ozonation were in the order of *Mycobacterium fortuitum* > poliovirus 1> *C. parapsilosis* > *E. coli* > *Salmonella typhimurium*. This group was suggested as a disinfection indicator since mycobacteria are probably the most resistant to physical and chemical agents of all the vegetative non spore forming bacteria (Lefford, 1983). They are highly resistant to drying, particularly if protected from sunlight, and less susceptible to UV light than many other species and relatively resistant to moderate heat. Some of the genera of *Mycobacterium* are pathogenic causing leprosy or tuberculosis while others are opportunistic pathogens. *Mycobacterium fortuitum* is an opportunistic pathogen.

Coliphages

Coliphages are a specific group of viruses which use coliform bacteria for host cells. Coliphages have been suggested to model the behavior of enteric viruses in disinfection, the fate of viruses in the environment, and indicate the presence of fecal pollution.

Coliphage contain only an outer protein coat (capsid) containing either DNA or RNA nucleic acid. The F+ specific coliphages (i.e. coliphage f2 or MS2) follow a procedure for host infection:

- adsorption of phage particles to host pili,
- ejection and penetration of phage nucleic acid,
- synthesis of phage macromolecules and subunits,
- assembly of mature phage particles, and
- lysis of the host cell and release of phages.

Coliphages have been found to seasonally correlate with enteric viruses in secondary sewage (Kott *et al.*, 1978) and river water (Geldenhuys and Pretorius, 1989). The coliphages behaved more similar to enteric viruses than other biological indicators like total coliforms, fecal coliforms, and fecal streptococci. Additionally, coliphages are present in wastewater in greater numbers than enteric viruses (Kott *et al.*, 1978; Grabow *et al.*, 1984) although the presence of coliphages were indicated even when no enteric viruses were found. Grabow and Coubrough (1986) suggested that coliphages were suitable indicators of human enteric viruses in water because coliphages outnumber viruses, they are at least as resistant, and coliphages are much more easily detected. However, some researches (IAWPRC, 1991; Havelaar and Pot-Hogeboom, 1988) feel that use of coliphages for viral indicators is limiting.

They argue that F+ specific coliphages can only be a measure of sewage pollution since there are relatively few coliphage excreted in human or animal feces. Additionally, it would be unlikely that coliphage would replace the traditional *E. coli* for indicator of sewage or fecal contamination.

Coliphages have been used to model the fate of viruses in the environment in terms of reaction to temperature, adsorption characteristics, and survival in wastewater treatment plants. Temperature was the most important factor for coliphage survival (Farrah, 1987). For increasing temperatures, the survival of coliphage was decreased (Farrah, 1987; Geldenhuys and Pretorius, 1989; Kutz and Gerba, 1988). Other factors of pH, presence of nitrates, turbidity, or hardness had little effect on coliphage survival. Additionally, the natural die-off of MS2 coliphage was similar to poliovirus (Farrah, 1987).

IAWPRC (1991) indicated that the f2 coliphage (or F+ specific coliphages) appears suitable for disinfection indicators based on the relative resistances of the f+ specific coliphages to chlorine and to UV light. It appears that f2 coliphage is more resistant to UV light than *E. coli*, poliovirus 2, poliovirus 3, echovirus 1, echovirus 11, coxsackievirus A9, coxsackievirus B1, reovirus 1, rotavirus SA11, and coliphage T3. Kott *et al.* (1974) determined that f2 and MS2 coliphages were more resistant to chlorine inactivation than E. coli and poliovirus 1. Sobsey *et al.* (1988) inactivated coliphages øX174 and MS2 with enteric viruses hepatitis A virus and coxsackievirus B5 with chlorine and chloramine. The MS2 coliphage was more resistant or had similar resistance to chlorine and chloramine as hepatitis A virus and coxsackievirus B5. On the other hand, coliphage øX174 was the least resistant of all showing the relative differences between coliphages. Moreover, MS2 coliphage was suggested as an indicator of virus inactivation by ozone (Malcolm Pirnie Inc. and HDR Engineering Inc., 1991).

Evison (1978) inactivated several enteric viruses, coliphages 185 and MS2, and *E. coli*. The ozone inactivation of the MS2 and 185 coliphages indicated that these coliphages were far more sensitive to ozonation than coxsackievirus B3, poliovirus 1, poliovirus 3, echovirus 1, and coxsackievirus B5. Evison (1978) demonstrated that certain types of ozone demand had little effect on the inactivation suggesting that the reaction of ozone with MS2 coliphage is very fast.

Finch and Fairbairn (1991) inactivated MS2 coliphage and poliovirus 3 paired in ozone demand free water. Their results showed that MS2 coliphage was significantly less resistant to ozonation than poliovirus 3. The MS2 coliphage had 1.6 logs higher inactivation than poliovirus 3.

2.4 DISINFECTION REGULATIONS

2.4.1 Canadian Regulations

The Guidelines for Canadian Drinking Water Quality (Health and Welfare Canada, 1989a) regulates utilities to provide drinking water with certain maximum contamination levels of microorganisms and chemicals. The ideal number of total coliform bacteria entering into the water distribution system would be zero detected in 100 mL. However, less than or equal to 10 CFU/100 mL of total coliforms (of which none are fecal) is acceptable.

There are currently no standards for viruses or *Giardia lamblia* in place. It is desirable, however, that no *Giardia* cysts or viruses are detected in the drinking water.

The maximum turbidity entering into the water distribution system must be less than or equal to 1 NTU. This may increase to less than or equal to 5 NTU only when it can be demonstrated that disinfection is not compromised.

The maximum concentration of THMs are 350 µg/L. This is currently under review and will probably lower to 50 µg/L in the foreseeable future.

2.4.2 US Regulations

The USEPA had recently updated the Safe Drinking Water Act (SDWA) to include the disinfection of all public water systems using any surface water and ground water directly influenced by surface water. This probably stems from the high rate of waterborne disease outbreaks found in utilities which treat surface or ground water by disinfection only or by utilities which have no treatment of groundwater (Craun, 1988). Surface water is considered to be all water open to the atmosphere and subject to surface runoff. Groundwater directly effected by surface water have significant occurrences of insects or other microorganisms and/or have sudden shifts caused by surface controlled phenomena such as shifts in turbidity, temperature, conductivity, or pH (Malcolm Pirnie Inc. and HDR Engineering Inc., 1991).

The maximum contaminant level goal for drinking water shall have zero *Giardia lamblia*, viruses, and *Legionella*. Furthermore, the turbidity and HPC bacteria must be as close to zero as possible (Pontius, 1990).

Additionally, disinfection alone is not always adequate for safe for removal of all waterborne pathogens. Filtration (or some other approved technology) is required, however, there is certain criteria for systems not filtering. For a utility to avoid filtering the water source, the following must be met (Malcolm Pirnie Inc. and HDR Engineering Inc., 1991):

- fecal coliforms must be ≤ 20 CFU/100 mL in 90% of the raw water samples
- total coliforms must be ≤ 100 CFU/100 mL in 90% of the raw water samples
- raw water turbidity must be ≤ 5 NTU
- must have disinfection adequate for 3 logs inactivation of *Giardia lamblia* and 4 logs inactivation of viruses based on Ct values
- disinfection residual must be ≥ 0.20 mg/L in the distribution system
- must have an effective watershed control program
- annual on-site inspection
- proven absence of waterborne disease incidents
- compliance with the total THM maximum contaminant level in the water distribution system
- compliance with the total coliform maximum contaminant level in the water distribution system

Utilities which can meet all the above criteria may be exempt from filtering. The total coliform rule suggests that the number of total coliforms should be less than 1 CFU/100 mL including fecal coliforms. The HPC will be maintained at less than 500 CFU/mL but these are effectively controlled by adequate disinfection residual (>0.2 mg/L). The maximum THM concentration of water must be 100 μg/L on a running annual average.

3.0 METHODS AND MATERIALS

3.1 INTRODUCTION

In this section, the methods and materials used for ozone disinfection of MS2 coliphage and HPC bacteria in North Saskatchewan River water are covered. This discussion includes methods for water parameter analysis, microbiology methods, ozone generation, and ozone disinfection. In addition, there is a discussion on the water characterization and several parameters that were important to ozone disinfection and how they were altered.

3.2 TEST WATER CHARACTERISTICS AND ANALYSIS

3.2.1 General

The test water used in this study was untreated North Saskatchewan River water obtained from the E.L. Smith Water Treatment Plant in Edmonton, Alberta. Nine samples were collected from E.L. Smith from November 14, 1991 to April 22, 1992. The majority of these samples were collected between January and April. Initial water analysis of the raw water samples are summarized in Table 3.1.

The water samples may be divided into two general categories relating to their quality. The first group of water samples were obtained while the North Saskatchewan River was covered with ice during winter. This group was comprised of water batches RW1801, RW2501, RW3101, RW0802, RW1302, and RW2702. The second group of samples were obtained during spring runoff and were water batches RW0304 and RW2204. Winter water samples were generally characterized by low turbidity, low DOC, low HPC and low total coliform concentrations while the spring runoff samples have considerably higher turbidity, DOC, HPC and total coliform concentrations. Sample RW1411 did not directly fit into either of these two grouping since it was collected before ice covered the river and after spring runoff.

The lower winter temperatures were partly responsible for the changing water quality observed in the North Saskatchewan River. During winter, no surface water enters into the river preventing terrestrial material from entering into the river. During spring runoff, the ice cover breaks and the water is contaminated with terrestrial humic material and bacteria. A portion of the river water is contributed from the groundwater. The groundwater is possibly a contributor of some hardness, alkalinity, and conductivity. During the winter, groundwater may contribute a greater portion of flow to the river resulting in slightly higher hardness, alkalinity, conductivity and pH. Monthly reports from The City of Edmonton Water Treatment Plants were reviewed to confirm the seasonal trends of water parameters during the time period from January 1989 to May 1992.

Table 3.1 - Water Sample Water Characteristics

Sample	DOC mg/L	pH	Turb NTU	Cond µS/cm	Alk meq/L	TH meq/L	TC CFU/dL	HPC CFU/mL	IOD mg/L
RW1411	7.040	8.23	6.0	325		3.52			
RW1801	1.210	8.15	2.9	385	2.92		24.6	1580	1.33
RW2501	2.044	8.19	2.4	278	2.94	3.65	38.5	813	0.80
RW3101	1.853	8.30	3.0	280	2.84	3.52	60.8	5100	1.28
RW0802	1.664	7.89	3.2	268	2.63	3.42	318	447	
RW1302	1.608	8.10	2.6	265	2.51	3.20	303	10,450	
RW2702	1.197	7.96	3.7	298	2.49	3.20	50	9530	0.63
RW0304	6.724	8.15	46.5	265	2.37	2.83	1370	837,000	3.54
RW2204	3.711	8.07	33.0	315	2.68	3.42	2231		1.13

3.2.2 pH

The pH of the water samples were measured using a Fisher pH meter (Model 925). The pH meter was checked each day the meter was used with standard buffer solution or recalibrated by two point calibration using standard buffer solutions. The pH measurements conformed to Standard Method 4500 (APHA, 1989).

The average raw water sample was 8.12 ± 0.13 pH units (see Table 3.1). An analysis of the monthly recorded data from E.L. Smith indicated that the pH changed seasonally from a low of 8.0 in January or February to a peak of about 8.4 in July or August.

3.2.3 Turbidity

The turbidity of the winter samples had an average of 3.0 ± 0.6 NTU which increased to 40 ± 10 NTU for the spring runoff samples. Measurement of the turbidity was analyzed using a Hach turbidity meter (Model 2100A) and according to Standard Method 2130 (APHA, 1989). The City of Edmonton

Monthly Reports indicated that the typical winter turbidities were between 2 and 6 NTU while the monthly average April turbidities were about 75 NTU. However, instantaneous peaks rose above 200 NTU. The sudden increase in turbidities found during the spring runoff are typical of the North Saskatchewan River and was caused by surface water washing debris, silt, and other organics into the river. In addition, turbidity spikes may also be caused by ice gouging the river banks suspending large amounts of inorganic silts and clays in the river.

3.2.4 Ozone Demand (OD)

The immediate ozone demand is defined as the mass of ozone which reacts immediately with compounds in the water. It was measured by subtracting the difference between the applied ozone dose and the measured ozone residual. Figure 3.1 illustrates an example of (immediate) ozone demand. As shown in this figure, the difference between the applied ozone dose and the measured ozone residual is the immediate ozone demand of the water.

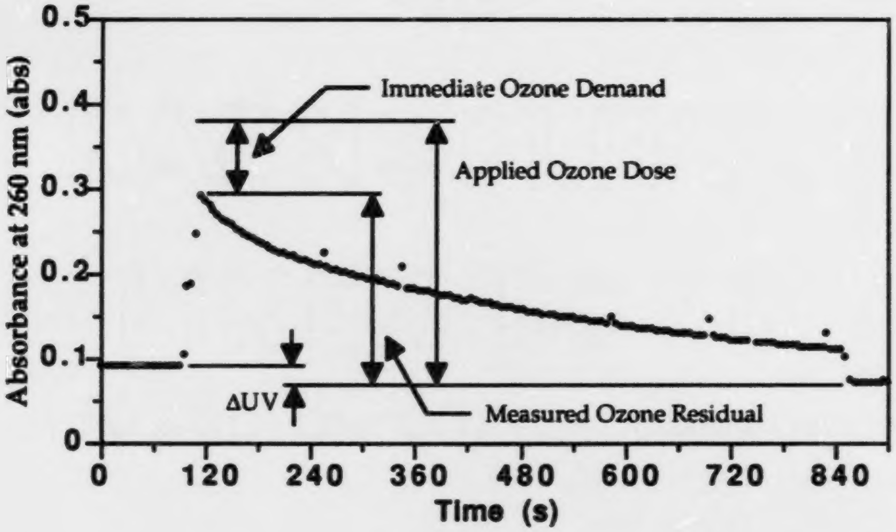


Figure 3.1 - Typical Example of Immediate Ozone Demand Measurement

Ozone demand was included in a supplement to Standard Methods (APHA, 1989) but this method was not available until most of the experiments were completed.

3.2.5 Total Hardness (TH)

Total hardness is the concentration of all polyvalent cations in solution. Magnesium and calcium hardness typically contribute the majority of the total hardness. The average total hardness of the samples was 3.34 ± 0.26 meq/L (milliequivalents per Liter) and was analyzed according to Standard Method 2340 (APHA, 1989). Review of the monthly reports indicated that the total hardness varies seasonally. The low period during the summer months was about 2.9 meq/L and peaks during the winter months was around 3.6 meq/L.

3.2.6 Temperature

Most of the experiments were conducted with the sample water at room temperature (22°C), however, several were conducted at lower temperatures (4°C). The actual monthly average temperature of the North Saskatchewan River water was about 8°C and varies seasonally between 1°C in the winter to 20°C during the summer. The room temperature was slightly higher than that of the maximum summer conditions.

3.2.7 Dissolved Organic Carbon (DOC)

The DOC of the raw water samples was measured for the various samples according to Standard Methods 5310 (APHA, 1989). The variation of the DOC was considerable between the winter and the spring runoff samples. The average winter sample had an average DOC of 1.596 ± 0.341 mg/L as C. The RW0304 sample had a DOC of 6.724 mg/L as C while the RW2204 sample had a DOC of 3.711 mg/L as C. The RW0304 sample was collected near or at the peak of spring runoff whereas the RW2204 sample was collected after the first spring runoff peaked. The monthly reports indicated the DOC of about 1.5 mg/L-C is typical for the winter months with sudden increases in April or May up to 7.6 mg/L-C. These sudden increases in DOC resulted from terrestrial humic materials washing into the river as the ice cover breaks and melts.

3.2.8 Conductivity

Conductivity is a general measurement of an aqueous solution to pass an electrical current and is directly proportional to total dissolved solids. Solutions with inorganic ionic compounds have higher conductivities than solutions with relatively fewer ions. The conductivity of the water samples was measured according to standard method 2510 (APHA, 1989). The average sample water conductivity was 287 ± 22 µS/cm.

Review of the Water Treatment Plant Monthly Reports indicated that the conductance varies seasonally peaking at about 375 µS/cm during the winter months and dropping to about 300 µS/cm during the summer months. Additionally, the conductance generally dips during April. This may be the result of a large amount of surface runoff diluting the river water.

3.2.9 Total Alkalinity

Total alkalinity is a measure of the acid neutralizing capacity of a water. The components measured are hydroxide ions, carbonate ions, and bicarbonate ions. The alkalinity of the water samples was measured according to standard method 2320 (APHA, 1989). The average alkalinity of the water samples was 2.67 ± 0.21 meq/L. Similar to total hardness, the total alkalinity had also varied seasonally. The alkalinity peaked at about 2.8 meq/L during the winter and dropped to about 2.5 meq/L during the summer.

3.2.10 Heterotrophic Plate Count (HPC) Bacteria

The HPC bacteria are a measure of the organic carbon utilizing bacteria endemic to the North Saskatchewan River. These bacteria were measured according to standard method 9215 (APHA, 1989). The concentrations varied considerably between the winter and spring runoff samples. During the winter, the HPC concentration was 10,000 CFU/mL or less but increased to greater than 800,000 CFU/mL during the spring runoff. The HPC bacteria microbial methods are discussed further in Section 3.4.

The Water Treatment Plants Monthly Reports indicated similar trends for the HPC population. During the winter months, the HPC concentration was about 10,000 CFU/mL or less, however, during spring runoff the HPC population increased considerably up to values near 1,000,000 CFU/mL.

The winter ice cover prevents any terrestrial HPC bacteria from entering the river and the colder temperatures limits the number and diversity of bacteria in the water. Generally, colder temperatures slow microbial metabolism.

3.2.11 Total Coliforms (TC)

Total coliforms concentration is a measure of the fecal pollution of the river. With the exception of *Klebsiella*, coliform bacteria normally live in the large intestine of warm blooded animals and are excreted in the feces of these animals. The total coliforms were assayed according to Standard Method 9222 (APHA, 1989). The total coliform microbial methods are further discussed in Section 3.4.

3.2.12 Color

The water samples were observed for color but not quantitatively analyzed. The presence of visually observable color would indicate the presence of humic materials in water. Only the RW0304 sample had a slight yellowish brown color even when filtered through a 0.45 μm polycarbonate membrane filter. The balance of the samples did not have any visually observable color.

The Water Treatment Plant Monthly Reports indicated that the color was lowest during the winter but suddenly jumps during the spring melt. It is most likely that the sudden increase in color was due to terrestrial humic material being washed into the river during the spring runoff. The increase in color coincides with the increase in DOC.

3.3 EXPERIMENTAL DESIGN AND ANALYSIS

3.3.1 Experimental Design for Bench Scale

Several water parameters were investigated for their effects on ozone disinfection of MS2 coliphage and HPC bacteria. Parameters tested were pH, turbidity, total hardness, DOC and temperature. These experiments were conducted in factorial design fashion since factorial designs have the advantage of efficiency and that the interaction terms between main effects can be calculated.

Most of the experiments were paired with both seeded MS2 coliphage and naturally occurring HPC bacteria. The experimental designs and levels are summarized in Tables 3.2 and 3.3 for the winter water batches and spring runoff batches, respectively. Included in these tables are the factorial design levels and the corresponding values used.

Table 3.2 - Experimental Design for Water Batches RW0802 and RW2702

Experiment	Factor			
	Ozone Dose (mg/L)	Turbidity (NTU)	T. Hardness (meq/L)	pH
RW0802	- (0.20)			- (7.06)
	+ (0.38)			- (7.06)
	- (0.20)			+ (7.94)
	+ (0.38)			+ (7.94)
	0 (0.30)			0 (7.43)
	0 (0.30)			0 (7.43)
RW2702		- (1.4)		- (7.04)
Exp #1		- (1.4)		+ (7.97)
		+ (14)		- (7.04)
		+ (14)		+ (7.97)
		0 (6.8)		0 (7.51)
		0 (6.8)		0 (7.51)
Exp #2		- (1.3)	- (3.19)	
		- (1.3)	+ (9.13)	
		+ (20)	- (3.19)	
		+ (20)	+ (9.13)	
		0 (7.3)	0 (6.22)	
		0 (7.3)	0 (6.22)	
Exp #3	- (0.04)			- (7.01)
	- (0.04)			+ (8.04)
	+ (0.22)			- (7.01)
	+ (0.22)			+ (8.04)
	0 (0.14)			0 (7.53)
	0 (0.14)			0 (7.53)
Exp #4	- (0.05)	- (2.5)		
	- (0.05)	+ (14)		
	+ (0.22)	- (2.5)		
	+ (0.22)	+ (14)		
	0 (0.13)	0 (6.4)		
	0 (0.13)	0 (6.4)		

Table 3.3 - Experimental Design for Water Batches RW0304 and RW2204

Experiment	Factor		
	Ozone Dose (mg/L)	DOC (mg/L)	Temperature (°C)
RW0304	- (0.05)	- (3.930)	
Exp #1	- (0.05)	+ (6.680)	
	+ (0.23)	- (3.930)	
	+ (0.23)	+ (6.680)	
	0 (0.13)	0 (4.731)	
	0 (0.13)	0 (4.731)	
Exp #3	- (0.22)	- (1.198)	
	- (0.22)	+ (5.196)	
	+ (0.45)	- (1.198)	
	+ (0.45)	+ (5.196)	
	+ (0.45)	+ (5.196)	
	0 (0.31)	0 (3.330)	
	0 (0.31)	0 (3.330)	
	0 (0.31)	0 (3.330)	
Exp #4	- (0.21)	- (1.498)	- (4)
	- (0.21)	- (1.498)	+ (22)
	- (0.21)	+ (4.851)	- (4)
	- (0.21)	+ (4.851)	+ (22)
	+ (0.43)	- (1.498)	- (4)
	+ (0.43)	- (1.498)	+ (22)
	+ (0.43)	+ (4.851)	- (4)
	+ (0.43)	+ (4.851)	+ (22)
	0 (0.33)	0 (2.908)	+ (22)
	0 (0.33)	0 (2.908)	+ (22)
	0 (0.33)	0 (2.908)	+ (22)
RW2204		- (1.619)	- (4)
Exp #2		- (1.619)	+ (22)
		+ (2.962)	- (4)
		+ (2.962)	+ (22)
		0 (2.218)	+ (22)
		0 (2.218)	+ (22)
Exp #3		- (1.340)	- (4)
		- (1.340)	+ (22)
		+ (2.882)	- (4)
		+ (2.882)	+ (22)
		0 (2.098)	+ (22)
		0 (2.098)	+ (22)

The factorial design bench scale experiments were generally divided into two experiments:

1) investigating the effects of pH, turbidity, and total hardness on the winter water samples; and

2) investigating the effects of DOC and temperature on spring runoff water.

Other bench scale experiments were ozone dose response of MS2 coliphage and HPC bacteria. These experiments were preliminary in nature but serve to illustrate the general inactivation characteristic of MS2 coliphage in raw surface water. Additionally, three pilot scale experiments were conducted using seeded MS2 coliphage, endemic HPC bacteria, and *Giardia muris* protozoa. The experiments with *G. muris* were conducted in conjunction with the research of Charles Labatiuk (Ph.D candidate).

3.3.2 Sample Preparation for Bench Scale

This section describes how the raw water samples were altered in pH, turbidity, total hardness, temperature and DOC (in conjunction with the applied ozone dose). Note that all of these factors were not altered for each experiment. For instance, only the pH and turbidity were altered for the 2x2 factorials involving pH and turbidity. The total hardness and DOC remained unchanged and the temperature was at room temperature (22°C).

pH

The pH of the raw water samples were adjusted by adding 6 M sulfuric acid or 1 M sodium hydroxide to the raw water sample until the desired pH was achieved. A pH meter continuously monitored the pH of the solution.

Turbidity

A bentonite clay slurry was prepared by mixing BDH bentonite clay with ozone demand free Milli-Q reagent water until the mixture was between 700 and 1000 NTU. This clay slurry was used to increase the raw water turbidity until the desired turbidity was reached. Note that the natural turbidity of the water was used as the low (-) level in the factorial design experiments RW2702 #1, RW2702 #3 and RW2702 #4.

Total Hardness

The total hardness of the raw water samples were adjusted by adding Fisherbrand calcium chloride until the desired total hardness was achieved.

The natural total hardness was used as the low (-) level in the factorial design experiment RW2702 #2.

Temperature

The cold temperature experiment samples were cooled in a fridge at 4°C. The samples were quickly removed from the fridge and ozonated before the samples could warm. The time difference from the fridge cooler to completion of the ozonation experiments were approximately 3 minutes or less.

DOC

The DOC was varied in some of the experiments by diluting the water samples with ozone demand free water. Note that this also resulted in a dilution of turbidity, pH, conductance, and alkalinity.

Applied Ozone Dose

The applied ozone dose was changed in the various experiments by adding different volumes of the ozone stock solution to the water samples.

3.3.3 Statistical Analysis

Analysis of Variance

Analysis of the results was done by one-way analysis of variance (ANOVA) as described by Davies (1979). The linear regression coefficient, β, was determined using a linear regression computer program Data Desk Professional. The total effect of a water parameter or interaction was found by:

$$TE = 2\beta 2^{m-n-1}$$

Where, β = regression coefficient
m = factorial design order
n = fraction of factorial design
TE = total effect

The mean square of the effect was found by:

$$MS = \frac{TE^2}{v2^{m-n}}$$

Where, v = degrees of freedom (typically 1)
MS = mean square of effect

Significant effects and interactions were determined by dividing the mean square of effect by the mean square of error and comparing it to tabulated (Box *et al.*, 1978) F-statistic values. Any mean square of effect greater than 5 percent significance level ($Pr \leq 0.05$) was considered significant while any effect greater than 1 percent significance level was considered highly significant.

Half Normal Plot

Half normal diagrams were plotted using the statistical computer package Systat 5.1. The total effect was plotted against the expected values. The main effects or interactions which fall on a straight line intersecting the origin were considered insignificant and due to random noise or chance. Those main effects and interactions which are skewed to the right of this line were considered significant (Daniel, 1959).

Mean Square of Error

The mean square of error was used to determine whether any of measured main effects or interactions from the factorial designs were statistically significant. The mean square of error were found individually for both MS2 coliphage and HPC inactivation experiments using replicated conditions.

3.4 MICROBIOLOGY METHODS

3.4.1 Media Preparation

All nutrient media and dilution buffers were prepared with Milli-Q (Millipore Corp.) reagent water and using Difco laboratory media. The laboratory chemicals were either BDH or Fisher Certified Reagents. After the media was mixed, the solution would be autoclaved for a minimum of 25 minutes. Petri dishes were poured from the hot sterile autoclaved media under the sterile laminar flow cabinet and allowed to set. The petri dishes would be stacked, wrapped in plastic and stored inverted at 4°C until use (usually within 1 week).

3.4.2 HPC Bacteria

HPC bacteria were enumerated according to standard method 9215 (APHA, 1989). The nutrient media was R2A agar, incubated at 20°C and counted on day 7 of incubation. R2A agar was used because it has less nutrients than HPC agar and it would less likely shock the natural HPC populations in the river. Additionally, 20°C incubation temperature was used since this temperature would much better represent the psychrophilic or mesophilic bacteria population in the river. Higher temperatures (i.e. 35°C) represent bacteria which would normally inhabit warm blooded animals.

3.4.3 Total Coliforms

Total coliform bacteria counts were determined according to Standard Method 9222 (APHA, 1989). The nutrient media was m-Endo LES agar and the plates were incubated for 24 hours at 35°C.

3.4.4 MS2 Coliphage

MS2 Coliphage Stock Suspension Preparation

A suspension of *Escherichia coli* (ATCC 15597) was inoculated into 5 mL of tryptone yeast extract broth (TYEB) and incubated overnight at 35°C. About 1 mL of existing MS2 coliphage stock was diluted in 0.1% peptone dilution bottles to 10^{-5} dilution. One mL of diluted MS2 plus 2 drops of *E. coli* plus 3 mL of top agar was plated onto each of the plates.

The plates were incubated overnight at 35°C with almost complete lysis of the *E. coli* host. A sterilized glass spreader was used to scrape off the top layer of MS2 coliphage and *E. coli* from each plate and transferred to a sterile 500 mL beaker and Teflon magnetic stir bar. The beaker of MS2 coliphage was mixed with 100 mL of sterile 0.05 M phosphate buffer, 0.4 g disodium EDTA, 0.05 g lysozyme and stirred at room temperature for about 2 hours. The contents were then transferred from the beaker to four sterile 50 mL centrifuge tubes with caps. The tubes were balanced and spun for 15 minutes at 12,000 RPM at

4°C temperature. The decant from the centrifuge tubes was filtered through a 0.45 μm membrane filter and stored at 4°C in a sterile bottle.

The stock suspension was assayed by the agar overlay method (see below). The titre of stock solution was 1 x 10^{12} PFU/mL.

Agar Overlay Method for Coliphage Enumeration

The agar overlay method for MS2 coliphage is an enumeration method where the phage is mixed with the host bacteria (*E. coli* ATCC 15597) in a bacteria nutrient agar and poured on top of sterile nutrient agar (Adams, 1959). The MS2 coliphage will infect the bacteria as the bacteria grow and multiply. Plaques form where the coliphage had infected a host bacteria.

One day prior to the MS2 coliphage disinfection experiment, the host bacteria were inoculated in 5 mL of TYEB and incubated at 35°C for 18 to 24 hours. It is important to have the bacteria in the log growth state for optimal infection from the MS2 coliphage.

Prior to enumeration of the MS2 coliphage, bottom agar plates were warmed for at least 20 minutes in the incubator at 35°C. The top agar were made liquid in a microwave and maintained in a liquid state in a warm water bath at 48.5°C.

For each sample, 3 replicates were made for each dilution. Optimal number of plaques on each plate ranged from 20 to 200. Lower than 20 gives rise to higher chances of error (by a Poisson distribution) and greater than 200 gives rise to enumeration problems. Dilutions were made in sterile 0.1% peptone water according to Standard Methods.

For each individual plate, one transfer pipet was used to withdraw the bacterial host from the *E. coli* overnight culture. One mL of top agar and two drops of host culture were mixed in the liquid top agar and then poured on the warmed bottom agar plates. The plates were allowed to solidify and then incubated at 35°C while inverted for 6 to 24 hours. The plaques could be easily seen and counted in this time period.

3.4.5 *Giardia muris*

Two pilot scale ozone inactivation experiments were conducted with *G. muris* cysts seeded into the raw surface water. The protozoan cysts were supplied by Dr. M. Belosevic of the University of Alberta. Evaluation of the *G. muris* was conducted in Dr. Belosevic's laboratory using methods described elsewhere (Labatiuk *et al.*, 1992).

3.5 OZONE METHODS

3.5.1 Bench Scale Tests

Ozone Generation and Measurement

Ozone gas was generated using a corona discharge generator (PCI Ozone Corp., Model C2P-9C-4) with extra dry oxygen feed gas. The ozone gas phase concentration was measured by UV absorption using an ozone monitor (PCI Ozone Corp., Model HC 12). Typical gas phase concentrations ranged from 6.2 to 6.5 percent ozone by weight. Ozone stock solution was prepared by bubbling the ozone gas through Milli-Q water. The ozone stock solution vessel was cooled in an ice water bath to increase the aqueous ozone concentration. The aqueous ozone stock solution concentration was measured by pipetting some of the solution into a 1.0 cm quartz cuvette and measuring the absorbance with a spectrophotometer (Milton-Roy, Model 601) at 260 nm. The absorbance coefficient used for calculations was $\varepsilon = 3300$ L/mol/cm. Typical ozone stock solution concentrations ranged between 40 and 43 mg/L. The aqueous ozone concentrations in the experimental trials were continuously monitored using a diode-array spectrophotometer (Hewlett-Packard, Model 8452A) at 260 nm. The water sample was pumped at 8 mL/min through a 1.0 cm path length 35 µL quartz flowcell.

Ozone residual was removed from the water samples using 1.0 mL of sterile 1.0 M sodium formate solution.

3.5.2 Pilot Scale Tests

Ozone Generation

Ozone gas for the pilot experiments was generated using a corona discharge generator (Welshbach Model T-180). Gas concentrations ranged between about 0.6 to 1.2% w/w ozone and flowrates were about 1.5 L/minute. The ozone gas was bubbled directly into raw North Saskatchewan River water in a semi-batch pilot scale ozone reactor.

Ozone Measurement

The ozone concentration in the sample water was measured by withdrawing samples from the reaction vessel at various time intervals and using the indigo method for determining the ozone concentration in these samples (APHA, 1989).

The absorbance of the indigo trisulfonate was measured using a UV/Vis spectrometer (Hewlett Packard Co., Model HP 8452A). Note that the indigo method is based on a molar absorbance coefficient of 2950 L/mol/cm at 254 nm which would underestimate the ozone concentration measured by direct UV absorbance of ozone at 260 nm wavelength and using $\varepsilon = 3300$ L/mol/cm.

Gaseous phase ozone concentration was measured by an ultraviolet spectrophotometer (PCI Ozone Corp., Model HC 12) for both the ozone feed gas and offgas concentration.

3.6 DISINFECTION PROCEDURE

3.6.1 Bench Scale Tests

Water samples were prepared according to the experimental designs found in Tables 3.2 and 3.3. MS2 coliphage stock was added to a 1 L sample and thoroughly mixed in the reaction vessel which consisted of a 1 L acid washed borosilicate Erlenmeyer flask and an acid washed Teflon stirbar. A 1.0 mL aliquot was removed for initial assay of MS2 coliphage and HPC bacteria. The ozone stock solution concentration was measured twice prior to disinfection and pipetted as a side-stream into the reaction vessel. The ozone residual was removed with 1.0 mL of sterile 1.0 M sodium formate solution. Both the initial and final microbial concentrations were assayed immediately after disinfection.

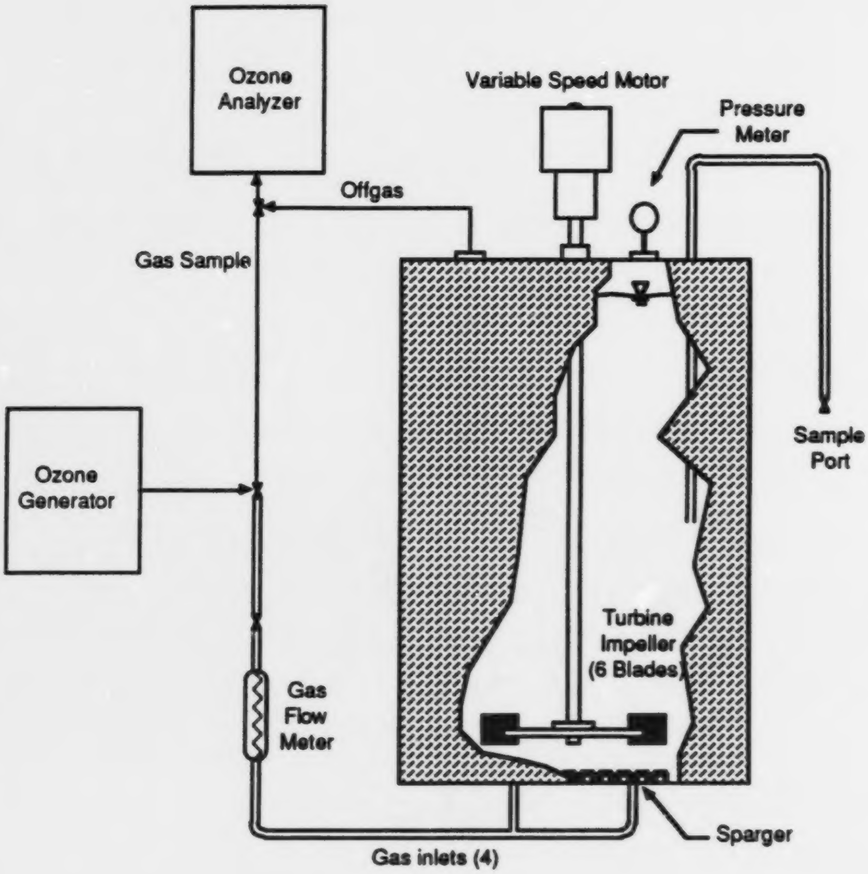
3.6.2 Pilot Scale Tests

Pilot tests were conducted using a semi-batch reactor vessel as shown in Figure 3.2. Details of this reactor have been reported elsewhere (Finch and Smith, 1989). MS2 coliphage and, in two trials, *G. muris* was seeded into the sample water prior to disinfection. Natural HPC bacteria were also monitored in one of the experiments. Ozone gas was fed through the sparger and mixed using the turbine impeller. At predetermined time intervals, samples were taken for microbial assays and ozone residual measurements. The samples for the microorganisms each contained 1 mL of sterile 1.0 M sodium formate to remove the ozone residual as the water was sampled. The samples for the ozone measurements each had 10 mL of indigo trisulfonate solution which reacted immediately upon sampling. The sample port is located about halfway through the reactor vessel and near to the side. Baffles are contained within the reaction vessel in order to achieve thorough mixing.

The MS2 coliphage and *G. muris* were seeded into solution in a port located on top of the vessel where the pressure meter may be found (see Figure 3.2). The ozone generator would be operating with the gas flowing to waste for at least 20 minutes prior to the experiment to give stable ozone generation. The offgas was monitored throughout the experiment for residual ozone concentration. The samples were processed immediately after the experiment.

3.7 GLASSWARE PREPARATION

All glassware used in the experiments were acid washed without any detergent. After the glassware was washed, the glassware was allowed to air dry and stored in a dry location. The pipet tips for the transfer of ozone from the stock solution to the samples were, however, made ozone demand free prior to the bench scale experiments.


Ozone Analyzer
Variable Speed Motor
Pressure Meter
Offgas
Gas Sample
Ozone Generator
Sample Port
Turbine Impeller (6 Blades)
Gas Flow Meter
Sparger
Gas inlets (4)

Specifics:

Reactor Hieght: 750 mm
Water Depth: 560 mm
Reactor Diameter: 500 mm
Length of Turbine Rod: 704 mm
Turbine Outer Diameter: 225 mm
Sparger Outer Diameter: 180 mm
Width of Baffles: 50 mm
Sparger Hole Density: 4650 - 1.5 mm ø holes per m^2
Materials: Stainless Steel and Teflon

Figure 3.2 - Diagram of Pilot Scale Reaction Vessel

4.0 RESULTS

4.1 INTRODUCTION

The experimental results are summarized and statistically analyzed in this section. Results from preliminary ozone dose response experiments, factorial designed disinfection experiments, pilot scale disinfection experiments, and ozone demand experiments are presented. The preliminary ozone dose response experiments were the first ozone bench scale experiments conducted and determined the effects of ozone on disinfection of MS2 coliphage. The factorial design experiments studied the effects of various water parameters on ozone disinfection. Pilot scale experiments were done to compare the inactivations of MS2 coliphage and HPC bacteria to those obtained in bench scale. Ozone demand experiments were also conducted to study parameters which may effect the ozone demand. Comparison and discussion of these results will be included in Section 5.0.

4.2 OZONE DOSE RESPONSE BENCH SCALE TESTS

Three bench scale MS2 coliphage disinfection experiments were completed to evaluate the ozone dose response of MS2 coliphage in untreated surface water. Samples RW1801, RW2501, and RW3101 were used in these experiments. Table 4.1 lists the inactivation results from these experiments.

Table 4.1 - Bench Scale Inactivation Results

Experiment	Applied Ozone (mg/L)	Residual Ozone (mg/L)	MS2 Inactivation (log)
RW1801	0.17	0.00	1.71
	0.36	0.00	3.50
	0.73	0.34	3.01
	0.78	0.21	3.71
	1.12	0.56	4.52
RW2501	0.04	0.00	0.22
	0.20	0.00	3.09
	0.44	0.00	2.97
	0.58	0.17	5.69
	0.82	0.19	6.04
RW3101	0.04	0.00	0.21
	0.21	0.00	5.07

The first experiment (RW1801) had relatively lower inactivations than the following two experiments for the same applied ozone dose. Also, relatively small inactivations were seen when a detectable ozone residual was measured. The ozone dose response data are plotted in Figure 4.1 for the three experiments. The experiments RW1801 had approximately 2 logs less inactivation than experiments RW2501 and RW3101 (with the exception of one relatively low inactivation in experiment RW2501).

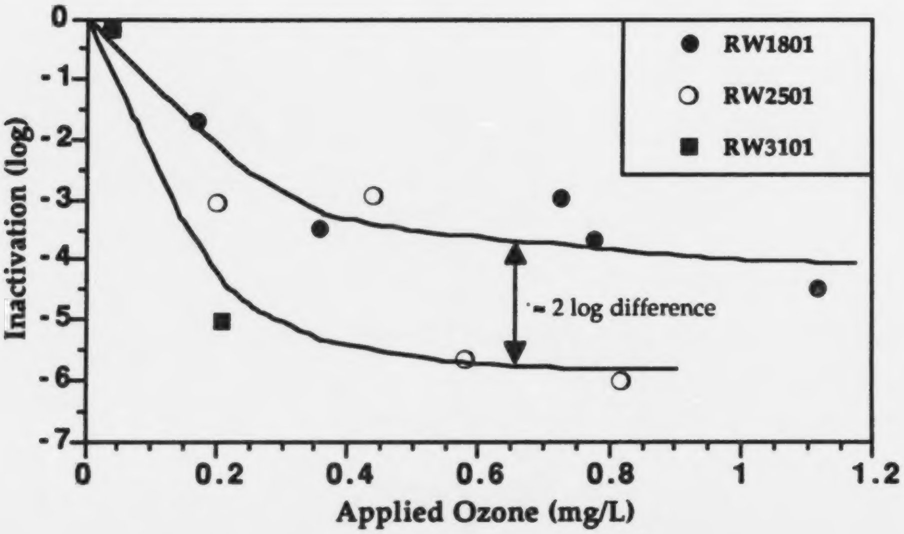


Figure 4.1 - Ozone Dose Response for Experiments RW1801, RW2501 and RW3101

This figure exhibits the tailing effect often seen with viral and bacterial inactivation curves (Kim *et al.*, 1980; Roy *et al.*, 1982; Finch and Smith, 1989; Havelaar *et al.*, 1990). There is also some evidence of a slightly delayed inactivation for the low applied ozone dose (0.04 mg/L) where the inactivation was slightly less than expected.

Figure 4.2 shows the inactivation of MS2 coliphage compared to the ozone residual at the end of the 20 second contact time. Again, there was about a 2 log difference between the inactivation of MS2 coliphage in experiment RW1801 and in RW2501 and RW3101. However, it is apparent that the consistently higher inactivations were obtained for any test runs where a residual had been produced. The test runs where zero ozone residual developed had widely variable inactivations ranging from 0.21 to 5.07 logs.

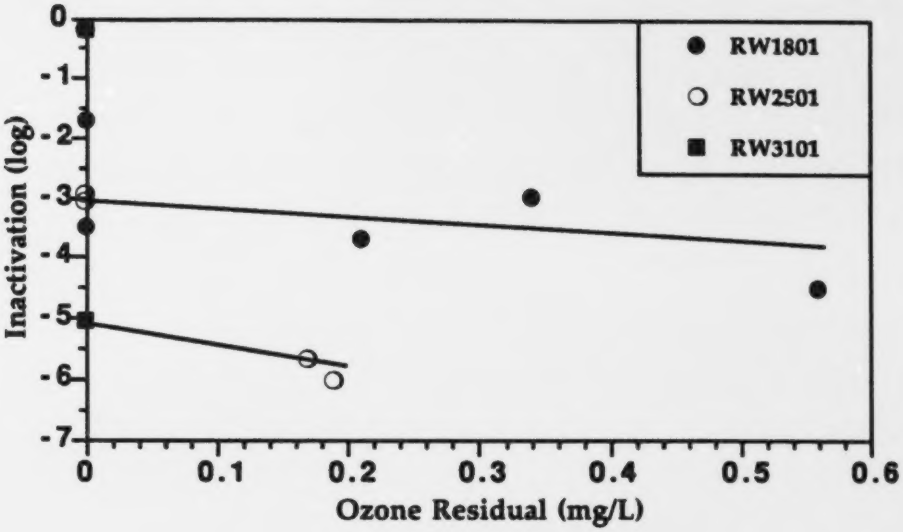


Figure 4.2 - Inactivation of MS2 Coliphage in Experiments RW1801, RW2501, and RW3101

4.3 BENCH SCALE FACTORIAL EXPERIMENTS

The bench scale factorial designs were set up to study the effects of natural water parameters on the disinfection of MS2 coliphage and HPC bacteria. The water parameters were pH, turbidity, total hardness, DOC and temperature. A series of ten factorial experiments were conducted to investigate the effects of these parameters.

The experiments were divided into two main groups since the water quality changed considerably from the winter to the spring runoff conditions. The first five experiments were done with water generally low in DOC and turbidity. This water was sampled during winter conditions when there was an ice cover over the river. This prevented most of the DOC from entering into the water. The other five factorial designed experiments were conducted when the spring runoff had started and was generally higher in DOC and turbidity. Moreover, the native HPC and TC populations had increased quite dramatically during the spring runoff. These later experiments were geared towards investigating the effect of DOC and temperature (and ozone dose) on the inactivation of MS2 coliphage and HPC bacteria. The first set of experiments were geared to investigate the effects of pH, turbidity, total hardness (and ozone dose) on the inactivation of MS2 coliphage and HPC bacteria.

Table 4.2 contains the factorial levels for each of the experiments. Table 4.3 summarizes the inactivation results from the experiments of water batches RW0802 and RW2702 (the winter water samples) while Table 4.4 shows the inactivation results from the experiments of water batches RW0304 and RW2204 (the spring runoff batches).

Table 4.2 - Levels For Water Batches RW0802, RW2702, RW0304, and RW0304

Factors	Experiment	Levels		
		(-)	(0)	(+)
Ozone dose (mg/L)	RW0802	0.20	0.30	0.38
pH (pH units)		7.94	7.43	7.06
Turbidity (NTU)	RW2702 Exp #1	1.4	6.8	14
pH (pH units)		7.04	7.51	7.97
Turbidity (NTU)	RW2702 Exp #2	1.3	7.3	20
Total Hardness (meq/L)		3.19	6.22	9.13
Ozone dose (mg/L)	RW2702 Exp #3	0.044	0.14	0.22
pH (pH units)		7.01	7.53	8.04
Ozone dose (mg/L)	RW2702 Exp #4	0.047	0.13	0.22
Turbidity (NTU)		2.5	6.4	14
Ozone dose (mg/L)	RW0304 Exp #1	0.045	0.13	0.23
DOC (mg/L as C)		3.930	4.731	6.680
Ozone dose (mg/L)	RW0304 Exp #3	0.22	0.31	0.45
DOC (mg/L as C)		1.918	3.330	5.196
Ozone dose (NTU)	RW0304 Exp #4	0.21	0.33	0.43
DOC (mg/L as C)		1.498	2.908	4.851
Temperature (°C)		4	-	22
DOC (mg/L as C)	RW2204 Exp #2	1.619	2.218	2.962
Temperature (°C)		4	-	22
DOC (mg/L as C)	RW2204 Exp #4	1.340	2.098	2.882
Temperature (°C)		4	-	22

The analysis of the factorial designs are summarized in Table 4.5. The results indicated that the most significant ($Pr \leq 0.05$) factors to ozone inactivation of MS2 coliphage and HPC bacteria were the applied ozone dose and the DOC. The applied ozone dose-DOC ratio was also highly significant ($Pr \leq 0.05$) to MS2 coliphage. The ozone reacted favorably with the DOC before significant MS2 coliphage inactivation occurred but when the ozone had reacted with all the faster components of the DOC, the slightly slower reactions between ozone and MS2 coliphage was prevalent. This situation only occurred in trials where the applied ozone dose was high enough to overcome the immediate ozone demand. For instance, all the trials with high DOC content in experiments RW0304 #3 and RW0304 #4 had inactivations of less than 1.3

logs for MS2 coliphage, but the trials with low DOC content and high applied ozone dose had inactivations greater than 3.6 logs for MS2 coliphage. A similar pattern was seen for the HPC bacteria, however, it appeared that the applied ozone dose was not high enough to satisfy all the competing reactions before reacting with the HPC bacteria.

Table 4.3 - Inactivation Results for Water Batches RW0802 and RW2702

	Factor				Inactivation (log units)	
Experiment	Ozone Dose	Turbidity	T. Hardness	pH	MS2	HPC
RW0802	-			-	5.73	
	+			-	6.20	
	-			+	5.70	
	+			+	6.00	
	0			0	5.51	
	0			0	6.10	
RW2702		-		-	5.07	2.17
Exp #1		-		+	5.40	1.76
		+		-	5.58	2.23
		+		+	5.55	1.84
		0		0	5.15	1.93
		0		0	5.20	2.20
Exp #2		-	-			1.87
		-	+			1.23
		+	-			1.74
		+	+			1.23
		0	0			1.56
		0	0			1.42
Exp #3	-			-	0.83	0.12
	-			+	0.51	0.17
	+			-	5.28	2.64
	+			+	5.32	1.85
	0			0	4.57	0.37
	0			0	4.16	0.33
Exp #4	-	-			0.31	
	-	+			0.29	1.06
	+	-			4.64	1.23
	+	+			5.58	1.25
	0	0				0.30
	0	0			2.49	0.32

A half-normal probability plot was prepared from the data of experiment RW0304 #4 involving a 2^3 factorial of ozone dose, DOC, and temperature (see Figure 4.3). A straight line passes through the origin and through the insignificant main effects and interactions. The significant effects and interaction are found to the right of the line. Only the ozone dose, DOC and ozone dose-DOC interaction were significant.

Table 4.4 - Inactivation Results for Water Batches RW0304 and RW2204

	Factor			Inactivation (log units)	
Experiment	Ozone Dose	DOC	Temperature	MS2	HPC
RW0304	-	-			0.19
Exp #1	-	+			0.00
	+	-			0.70
	+	+			0.00
	0	0			0.85
	0	0			1.33
Exp #3	-	-		0.78	0.41
	-	+		0.15	0.08
	+	-		3.59	1.09
	+	+		0.67	0.38
	+	+		0.45	
	0	0		0.77	
	0	0		0.73	
Exp #4	-	-	-	0.87	
	-	-	+	0.51	
	-	+	-	0.52	
	-	+	+	0.55	
	+	-	-	4.05	
	+	-	+	4.57	
	+	+	-	0.78	
	+	+	+	1.30	
	0	0	+	1.17	
	0	0	+	1.42	
RW2204		-	-	5.38	
Exp #2		-	+	5.80	3.02
		+	-	5.14	2.53
		+	+	6.36	2.09
		0	+	4.99	2.82
		0	+	5.28	2.30
Exp #3		-	-	6.59	3.65
		-	+	7.33	3.21
		+	-	6.17	3.21
		+	+	5.59	3.17
		0	+	6.39	3.06
		0	+	6.33	3.48

Although the factors of pH and turbidity were not found to be important to the inactivation of MS2 coliphage (see Table 4.5), the temperature may be significant. MS2 Coliphage inactivation in experiment RW2204 #2 was affected by the temperature and the MS2 coliphage inactivation in experiment RW2204 #3 was affected by the DOC-temperature interaction although this same interaction was not significant in the previous experiment. However, the 2x3 factorial experiment RW0304 #4 was not affected by the temperature. The ozone dose-DOC interaction was significant in both the RW0304 #3 and RW0304 #4 experiments.

Table 4.5 - ANOVA Results for MS2 Coliphage and HPC Bacteria Inactivation

Experiment	Factor	$\frac{MS}{MS_d}$ HPC	Significance (Pr ≤ 5%)	$\frac{MS}{MS_d}$ MS2	Significance (Pr ≤ 5%)
RW0802	A	-		3.34	
	D	-		0.312	
	AD	-		0.156	
RW2702 Exp 1	B	2.88		0.512	
	D	0.090		2.36	
	BD	0.000		0.713	
RW2702 Exp 2	B	0.072		-	
	C	5.98	Yes	-	
	BC	0.072		-	
RW2702 Exp 3	A	79.6	Highly	476	Highly
	D	2.48		0.445	
	AD	3.18		0.713	
RW2702 Exp 4	A	0.325		515	Highly
	B	0.000		4.71	
	AB	-		5.03	
RW0304 Exp 1	A	1.09		-	
	E	4.08		-	
	AE	1.26		-	
RW0304 Exp 3	A	4.33		57.7	Highly
	E	4.88		74.5	Highly
	AE	0.650		32.0	Yes
RW0304 Exp 4	A	-		189	Highly
	E	-		130	Highly
	F	-		1.40	
	AE	-		108	Highly
	AF	-		5.21	
	EF	-		0.422	
	AEF	-		0.422	
RW2204 Exp 2	E	7.80	Yes	0.578	
	F	1.75		15.0	Yes
	EF	-		3.56	
RW2204 Exp 3	E	1.04		25.9	Yes
	F	1.04		0.133	
	EF	0.722		9.70	Yes

A = Applied ozone dose B = Turbidity MS_d = 0.04493 with 8 degrees of freedom (For MS2)
C = Total hardness D = pH MS_d = 0.05507 with 7 degrees of freedom (For HPC)
E = DOC F = Temperature

It was interesting to note that very few parameters were found important to the inactivation of HPC bacteria. The ozone dose was highly significant in the RW2702 #3 experiment and the effect of DOC on ozone disinfection was significant in experiment RW2204 #2. Also, the total hardness may be significant in experiment RW2702 #2. Factors that were not significant to

HPC inactivation were the pH, turbidity, and temperature. Additionally, none of the interactions were significant to the inactivation of HPC bacteria.

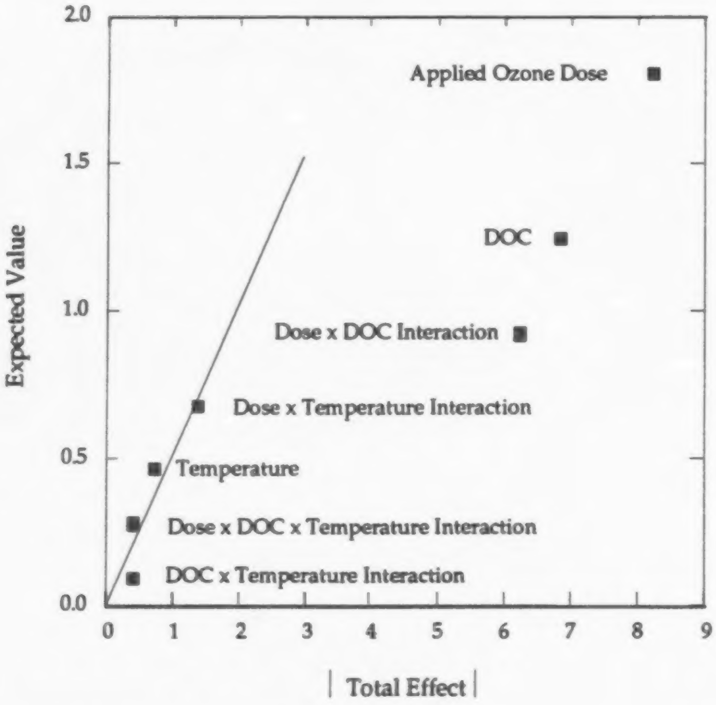


Figure 4.3 - Half Normal Probability Plot of Experiment RW0304 #4

4.4 PILOT SCALE TESTS

Three pilot scale tests were conducted with the semi-batch stirred tank reactor. The samples were raw North Saskatchewan River Water obtained from the E.L. Smith Water Treatment Plant. The first experiment (RW1411) was seeded with *Giardia muris*; the second experiment was conducted with seeded *G. muris* and MS2 coliphage; and the third experiment had seeded MS2 coliphage and native HPC bacteria.

Table 4.6 summarizes the results for the applied ozone dose, ozone residual, sample time, Ct calculation, and resulting inactivations for the pilot scale experiments. The Ct was estimated by multiplying the sample time by one-half the ozone concentration. This gives the average contact time and is similar to the method used in the AWWA Guidance Manual for Compliance with the Filtration and Disinfection Requirements (Malcolm Pirnie Inc. and HDR Engineering Inc., 1991).

Table 4.6 - Pilot Test Results for Experiments RW1411, RW3101, and RW1302

Exper.	Applied Ozone mg/L	Residual Ozone mg/L	Sample Time min	Est. Ct mg-min/L	MS2 Kill logs	HPC Kill logs	*G. muris* Kill logs
RW1411		0.38	3.17	0.60			1.6
		0.70	6.25	2.19			3.1
RW3101	0.00	0.00	0.00	0.00	0.00		
	1.47	0.21	4.00	0.42	5.79		2.7
	2.44	0.35	6.00	1.05	6.74		3.6
	2.93	0.54	8.00	2.16	5.77		
	3.67	0.80	10.0	4.00			5.0
	4.95	1.23	13.5	8.30			5.0
RW1302	0.00	0.00	0.00	0.00	0.00	0.00	
	0.14	0.00	1.83	0.00	3.62	0.00	
	0.28	0.02	3.58	0.04	5.39	0.45	
	0.34	0.05	4.35	0.11		1.08	
	0.45	0.09	5.67	0.26	5.36	1.21	

It appears that the MS2 coliphage were readily inactivated since any detectable ozone residual produced greater than 5 logs of MS2 inactivation. This suggests that any achievable Ct in this ozone reactor could result in greater than 5 logs MS2 coliphage inactivation. Conversely, the HPC bacteria and *G. muris* both had considerably less inactivation than the MS2 coliphage. Figure 4.4 shows the inactivation of the MS2, HPC, and *G. muris* with ozone residual. The MS2 coliphage inactivation was at least 3 to 6 logs higher than for either *G. muris* or HPC bacteria. This suggests that MS2 coliphage is much more sensitive to ozonation than either the HPC bacteria or protozoan cysts.

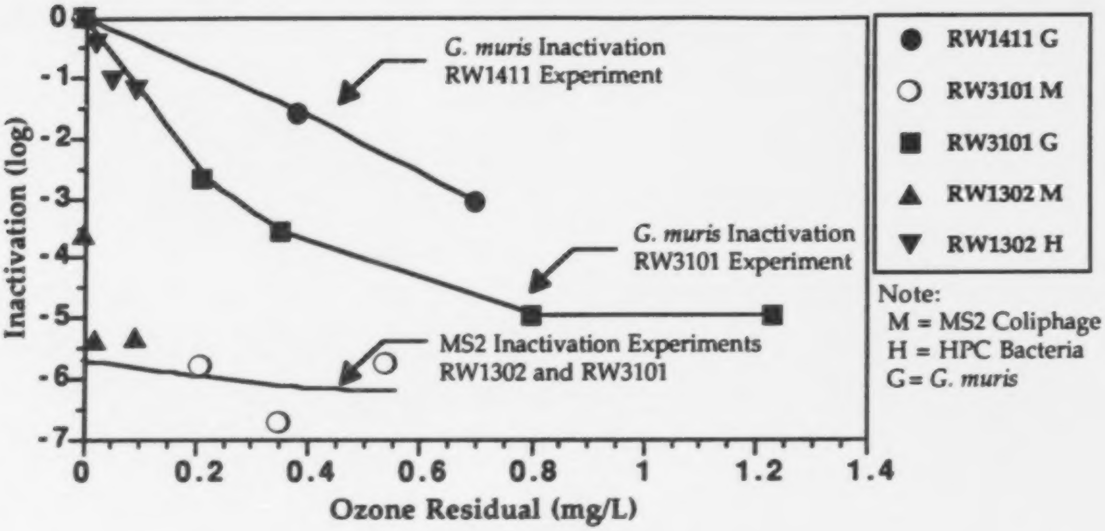


Figure 4.4 - Inactivation Results of Pilot Scale Experiments

4.5 BENCH SCALE OZONE DEMAND

Two ozone demand bench scale tests were conducted on raw North Saskatchewan River water to determine the effects of organic carbon concentration, type of organic carbon (i.e. suspended or dissolved), and temperature on the immediate ozone demand. To distinguish between the suspended and dissolved organic carbon, some of the samples were filtered through a 0.45 µm membrane filter.

Table 4.7 summarizes the results from all the replicated ozone demand tests. The table also includes the test conditions (i.e. temperature, filtered, etc.) and the sample deviation of the replicates. These replicates were used to obtain the mean square of error for the ozone demand factorial designs. The mean square of error was estimated to be 0.01840 with 13 degrees of freedom.

Several of the replicates were done with Milli-Q reagent water. Some of these trials had negative ozone demands indicating that the measured ozone residual was greater than applied. This suggests that the mixing was sometimes not homogeneous in the reaction flask.

Table 4.7 - Ozone Demands of Various Water Batches

Experiment	DOC (mg/L)	Temperature (°C)	Filtered	IOD (mg/L)	Ave. ± SD
RW1801	1.210	22	No	1.24	
	1.210	22	No	1.41	1.33 ± 0.12
RW2501	2.040	22	No	1.03	
	2.040	22	No	0.58	0.80 ± 0.32
RW3101	1.616	22	No	1.21	
	1.616	22	No	1.35	1.28 ± 0.10
RW2702	1.197	22	No	0.64	
	1.197	22	No	0.61	0.63 ± 0.02
RW0304	4.731	22	No	2.40	
	4.731	22	No	2.17	2.28 ± 0.16
	3.930	22	No	2.30	
	3.930	22	No	2.27	2.28 ± 0.02
	6.680	22	No	3.55	
	6.680	22	No	3.53	3.54 ± 0.01
Milli-Q		22		-0.15	
		22		-0.00	
		22		0.01	
		22		-0.32	
		22		-0.04	-0.10 ± 0.13
RW2204		22	Yes	1.84	
		22	Yes	1.92	1.88 ± 0.06
	3.202	22	No	1.13	
	3.202	22	No	1.13	1.13 ± 0.00

Table 4.8 contains the results from the two 2^3 factorial design experiments on RW2204. The results are analyzed by one-way analysis of variance and are included in Table 4.9.

Table 4.8 - Factorial Design and Results for Ozone Demand Experiments

Parameter			Results	
Organic Carbon Concentration (mg/L-C)	Temperature (°C)	Filtered	Ozone Demand Exp. 1 (mg/L)	Ozone Demand Exp. 2 (mg/L)
-	-	-	0.27	0.03
-	-	+	0.36	-0.03
-	+	-	0.74	0.64
-	+	+	0.62	0.50
+	-	-	0.64	0.23
+	-	+	0.44	0.41
+	+	-	1.20	1.04
+	+	+	1.20	1.04

Table 4.9 - ANOVA Results for Ozone Demand Experiments

Experiment	Factor	MS	MS/MS_d	Significance ($Pr \leq 0.05$)
Exp 1	A	0.512	29.2	Yes
	B	0.284	16.2	Yes
	C	0.007	0.400	
	AB	0.042	2.40	
	AC	0.000	0.000	
	BC	0.004	0.228	
	ABC	0.020	1.14	
Exp 2	A	0.824	47.1	Yes
	B	0.315	18.0	Yes
	C	0.000	0.000	
	AB	0.012	0.685	
	AC	0.009	0.514	
	BC	0.017	0.971	
	ABC	0.002	0.114	

A = Organic Carbon Content
B = Temperature
C = Filtered
MS_d = 0.01840 with 13 degrees of freedom

The results of the ANOVA indicate that the DOC content and the temperature were significant to the ozone demand. The higher DOC concentrations produced higher ozone demands. Furthermore, the ozone demand was significantly reduced when the temperature decreased from 22 to 4°C.

The half normal plots, shown in Figures 4.5 and 4.6, illustrate the same observations shown in Table 4.9. Again, the DOC concentration and the temperature were the most significant factors affecting immediate ozone demand. These factors are skewed to the right of the line which passes through the origin. The fact that some of these samples were filtered had no bearing on the ozone demand and neither did any of the interactions.

Several ozone demand experiments using RW2702 were conducted but using ozone doses of about 0.20 mg/L (this was considerably lower than the applied ozone doses seen in Table 4.7). The ozone demands of these trials were considerably lower than for higher applied dosage ozone demand experiments. Apparently, the ozone demands correlated with the applied ozone dose indicating that the immediate ozone demand was highly dependant on reaction kinetics. Larger ozone doses caused considerably higher ozone demands and compounds which would not normally react with ozone at low dosages would react at the higher ozone concentrations.

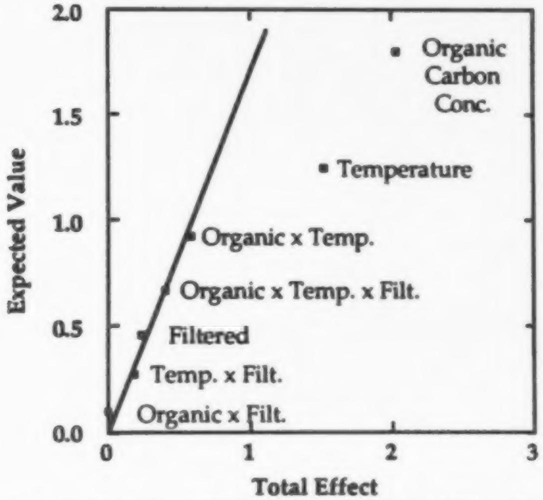


Figure 4.5 - Half Normal Plot of Ozone Demand Experiment #1

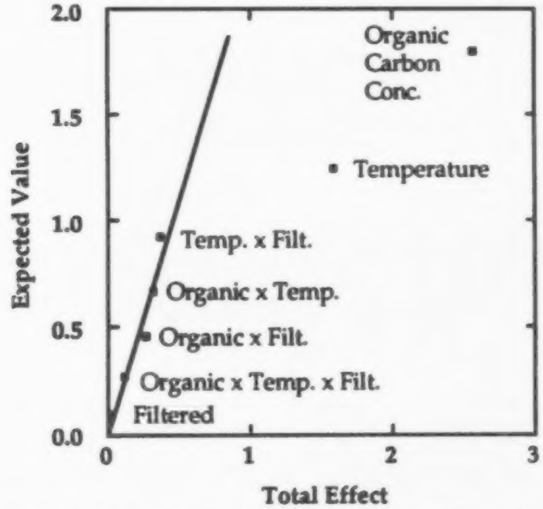


Figure 4.6 - Half Normal Plot of Ozone Demand Experiment #2

5.0 DISCUSSION

5.1 FACTORS AFFECTING MS2 COLIPHAGE AND HPC BACTERIA INACTIVATION

The results of the ANOVA in Table 4.5 indicated that the most significant ($Pr \leq 0.05$) factors to ozone inactivation of MS2 coliphage and HPC bacteria were the applied ozone dose and the DOC. Also, the applied ozone dose-DOC ratio was significant ($Pr \leq 0.05$) to MS2 coliphage but not to HPC bacteria. The effect of DOC was extremely important since a portion of the applied ozone reacted directly with the DOC before disinfecting a significant number of bacteria or viruses. In the winter water batches, the DOC was such that significant inactivation could be achieved at relatively low applied ozone doses. Experiments with spring runoff water showed that, for the same ozone doses as the winter samples, relatively low inactivations of MS2 coliphage and HPC bacteria had occurred. Figure 5.1 illustrates the relative difference between the inactivations for winter batch water (low DOC) and spring runoff water (high DOC). Low DOC water had approximately 5 logs more inactivation than the high DOC water.

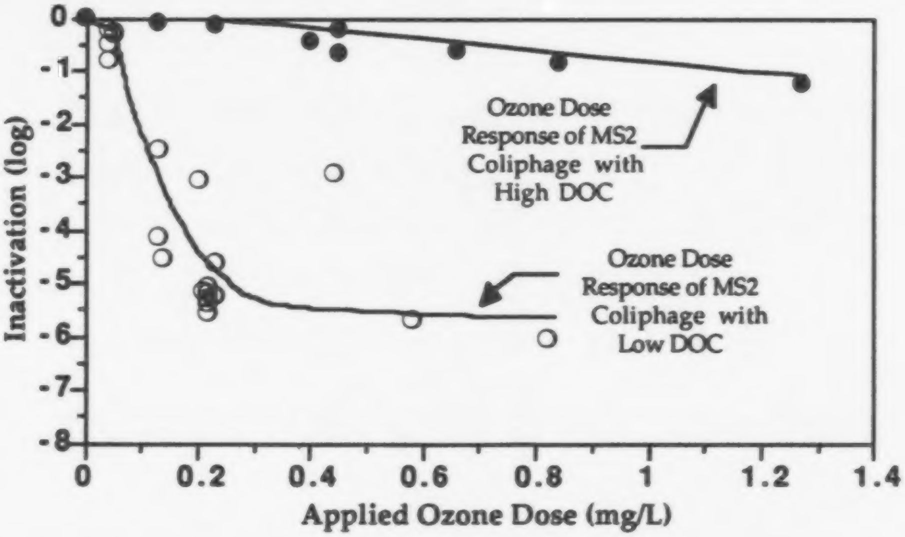


Figure 5.1 - MS2 Coliphage Dose Response for Low and High DOC Water Batches

The effect of the DOC is that the ozone preferred to reacted with certain components of DOC rather than MS2 coliphage. For the same applied ozone dose, less ozone would be available for inactivation implying that a higher ozone dose would be required for the same level of inactivation. The speed at which viruses are inactivated in low DOC water is very fast. Many of the experiments never developed an ozone residual, yet there was considerable MS2 coliphage inactivation. As shown in Figure 5.2, an ozone dose of 0.22 mg/L reacted immediately in the water without producing a residual. An immediate drop of absorbance was seen in this experiment indicating that the ozone (or ozone by-products) reacted with the compounds which caused the background absorbance in the water. Also in this experiment, there was 5.55 logs of MS2 coliphage inactivation even without a measurable ozone residual. This fast rate of inactivations were observed by other researchers (Kim *et al.*, 1980) where the f2 coliphage reacted within 5 seconds or less. However, in the spring runoff batches the ozone reacted preferentially with the DOC leaving less ozone for MS2 coliphage inactivation. Considerably lower MS2 coliphage inactivations were observed for trials with high DOC.

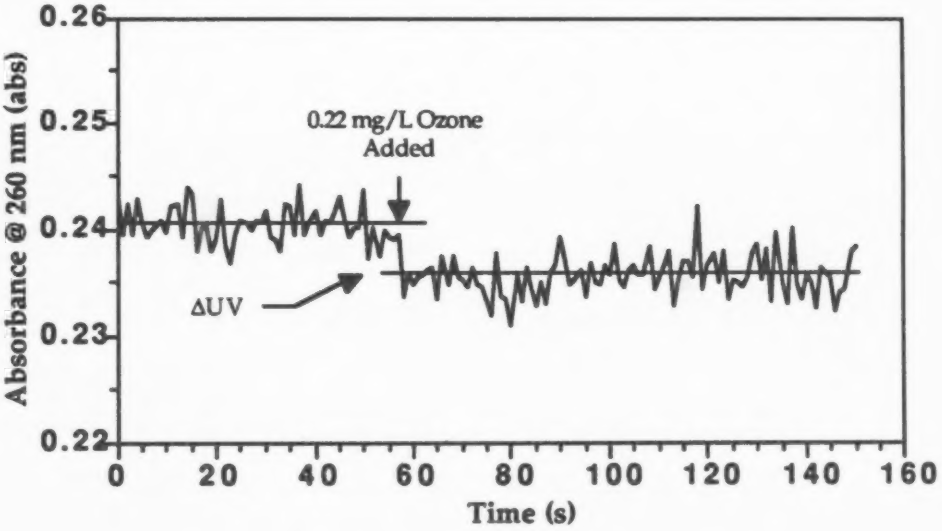


Figure 5.2 - Inactivation Trial Showing Zero Residual

The ozone dose-DOC interaction was also shown to be significant to the inactivation of MS2 coliphage. This was a result of the changing reaction kinetics of the DOC during ozonation. The ozone reacted favorably with the DOC before significant MS2 coliphage inactivation but when the ozone had reacted with all the faster components of the DOC, the slightly slower reactions between ozone and MS2 coliphage were prevalent. This situation occurred in trials where the applied ozone dose was high enough to overcome the immediate ozone demand. For instance, all the trials with high DOC content in experiments RW0304 #3 and RW0304 #4 had inactivations of less than 1.3 logs regardless of ozone dose, but the trials with low DOC content and high applied ozone dose had inactivations greater than 3.6 logs. A similar pattern was seen for the HPC bacteria, however, it appeared that the applied ozone dose was not high enough to satisfy all the competing DOC reactions and the reaction of ozone with HPC bacteria was much slower.

The other factors of pH and turbidity did not appear to affect the inactivation of MS2 coliphage or HPC bacteria. Higher pH waters could have lower inactivation than lower pH water because the ozone will stay in solution longer at a higher concentration at low pH. However, ozone disinfection has been reported to be relatively insensitive to pH in the range normally found in natural water (SDWC, 1980). Additionally, autodecomposition of ozone is either only minimally or not important to the ozone disinfection of acid fast bacteria, yeast, Hepatitis A Virus or Rotavirus (Vaughn *et al.*, 1987; Vaughn *et al.*, 1990; Farooq *et al.*, 1977a). These researchers concluded that the ozone residual is more important to ozone caused inactivation than is the autodecomposition rate of ozone. However, Roy *et al.* (1982) concluded that ozone disinfection was greater at a higher pH for poliovirus 1 and echovirus 1. The effect of pH in this study would be secondary for the water tested since the immediate ozone demand had a dominant effect on the ozone inactivations.

Natural turbidity in water may be in the form of organic or inorganic suspended matter. Organic turbidity may compete with the ozone which would reduce the ozone inactivation of viruses and bacteria (Hoff and Akin, 1986; Foster *et al.*, 1980). Hoff and Akin (1986) concluded that inorganic turbidity did not affect inactivation of viruses or bacteria. However, inorganic turbidity protected coliphages T2, f2, and MS2 from ozone inactivation (Kaneko, 1989; Boyce *et al.*, 1981). Ozonation of raw surface waters may, in some cases, cause microflocculation of the water by creating more polar particles that are richer in oxygen and poorer in carbon double bonds (Maier, 1984; Singer and Chang, 1988). These particles may either adsorb on the turbidity causing particles to flocculate or by precipitation of the dissolved organic matter by creating less soluble reaction byproducts (Maier, 1984). In experiment RW2702 #2, the effects of turbidity and total hardness on ozone disinfection were investigated. It was expected that increasing the total hardness and turbidity would induce microflocculation and perhaps affect ozone disinfection. The total hardness appeared to protect the bacteria from ozonation.

Temperature will also increase the rate of ozone autodecomposition in water. With higher water temperatures the ozone half life will be shorter because the ozone will react faster in warmer temperatures. By induction, this would suggest that warmer temperatures should have lower inactivations. Conversely, cooler temperatures also decrease chemical reaction rates. If the reaction rate of viruses decreases at a less rate than DOC, then the effect of lowering the temperature may not be significant. On the other hand, if the reaction rate of viruses slows down at a faster rate than DOC, then lowering the temperature would make less ozone available for disinfection. In general, the accepted "Rule of Thumb" is that a 10°C decrease in temperature will reduce the reaction rate by half for all reactions.

The reaction rate of ozone and viruses is very rapid (Kim *et al.*, 1980). Even for a considerable decrease in temperature, the effect of temperature on the disinfection rate may not be easily measurable. Higher temperatures produced greater inactivation of MS2 coliphage. This suggests that the viruses may be more susceptible to ozone at higher temperatures or that the reaction rate of ozone with DOC increases at a slower rate (with temperature) than the reaction rate of ozone with viruses. The ozone inactivation of poliovirus 1, amoeba cysts, *Mycobacterium fortuitum*, coliphage T2, and *E. coli* increased with temperature (Wickramanayake and Sproul, 1988; Farooq *et al.*, 1977b; Katzenelson *et al.*, 1974). However, the ozone inactivation of hepatitis A virus and *E. coli* increased for lower temperatures (Herbold *et al.*, 1989). Moreover, Herbold *et al.* (1989) did not find that ozone inactivation of poliovirus 1 was affected by a change in temperature. Roy *et al.* (1982) concluded from their experiments with enteric viruses that a temperature increase results in a decrease in resistance to ozone inactivation.

The effects of the temperature, DOC, pH, (among others) may be related to the reaction kinetics of ozone in water. Factors such as pH and temperature may increase the autodecomposition of ozone in solution limiting the ozone utilized by the microorganisms. These effects are also confounded by the effects of competing reactions (i.e. DOC), clumping of the organisms, or effects of the many other factors which may effect the ozone in water (i.e. light, carbonate alkalinity, etc.). For instance, lower temperatures may have higher ozone inactivation for the resistant *G. muris* cysts because the lower temperatures maintain higher ozone residuals in solution for longer periods of time which allows a greater amount of ozone utilized by the cysts (Finch *et al.*, 1992; Wickramanayake and Sproul, 1988). Higher temperatures lower the utilized ozone by increasing the relative ozone reaction rate of other constituents in the water. For viruses, the amount of utilized ozone required for inactivation is smaller so that the temperature would have less of an effect. Ozone inactivation of viruses increased with temperature suggesting that the relative reaction kinetics of ozone may be greater with viruses (than with the competing reaction mechanisms) at higher temperatures. At lower temperatures, the reaction kinetics of the other mechanisms (i.e. competing reactions of DOC or hydroxyl ions) are more pronounced resulting in lower inactivation rate.

5.2 PAIRED DATA INACTIVATION

The MS2 coliphage and the HPC bacteria were paired in most experiments but the difference between the inactivation of the two microorganisms only became apparent for applied ozone dosages of greater than 0.85 mg/L (see Figure 5.3). For these dosages, the inactivation of MS2 coliphage was approximately 3 logs higher than HPC bacteria. Some of the pairs had considerably lower than 3 logs difference between the MS2 and HPC inactivations. The smaller differences seen in Figure 5.3 for applied ozone dosages of less than 0.50 mg/L also resulted from the relative change in reaction kinetics. Before spring runoff, the ozone reacted generally faster with the MS2 coliphage than the DOC or HPC bacteria. Samples taken during spring runoff (with high DOC) had some organic matter which reacted faster with ozone than ozone reacted with MS2 coliphage and HPC bacteria. This resulted in some of the inactivations of the MS2 coliphage and HPC bacteria having less difference at the lower applied ozone doses. The relative difference between the MS2 coliphage and HPC bacteria was more consistent at higher applied ozone dosages because the competing reactions had generally been satisfied and more ozone was available for inactivation of MS2 coliphage and HPC bacteria. The average mean difference between the MS2 coliphage and HPC bacteria was 2.8 logs for all the experimental conditions of the ozone disinfection experiments.

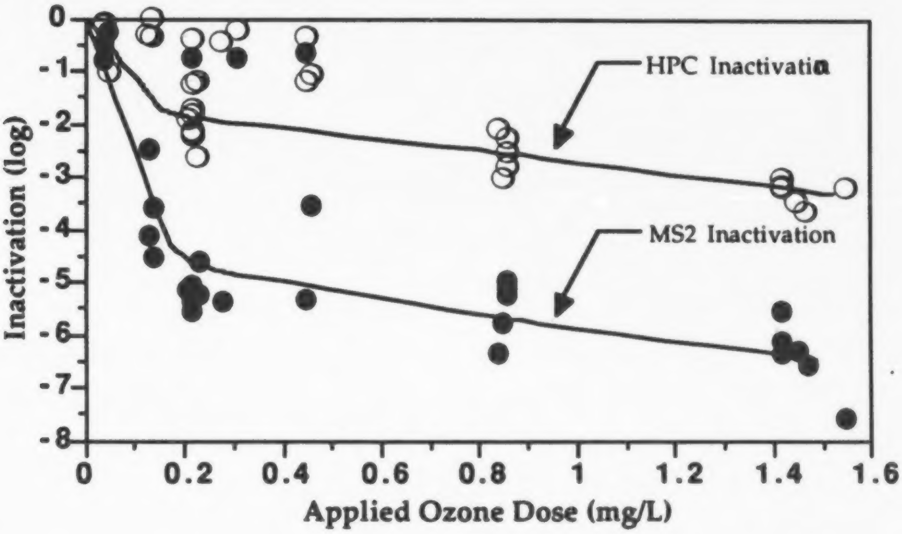


Figure 5.3 - Paired Inactivation of MS2 Coliphage and HPC Bacteria

5.3 EFFECT OF OZONE RESIDUAL

Generally, applied ozone dosages of greater than 0.85 mg/L produced a measurable residual at the end of the 30 s contact time in the bench scale tests. It may be seen (see Figure 5.4) that any ozone residual produced inactivations greater than 4 logs MS2 coliphage and greater than 2 logs of HPC bacteria. An ozone residual of 0.20 mg/L produced inactivations greater than 5 logs of MS2 coliphage and greater than 3 logs of HPC bacteria. Similar findings were observed by other researchers for coliphage and HPC bacteria inactivations. Evison (1978) inactivated 5 logs of coliphage 185 with 0.08 mg/L applied ozone within 20 min in phosphate buffer. Kim *et al.* (1980) had found that 0.085 mg/L ozone residual inactivated greater than 6 logs of f2 coliphage after 30 s contact time in phosphate buffer. They also report that much of the f2 coliphage was inactivated within the first 5 seconds. Wolfe *et al.* (1989)

ozonated MS2 coliphage, f2 coliphage, *E. coli* and HPC bacteria in raw surface water. The inactivations of MS2 and f2 coliphage were almost identical to each other indicating similar resistances and clumping characteristics between MS2 and f2 coliphage. Greater than 7 logs inactivation of coliphage MS2 and f2 were obtained for ozone residuals of at least 0.24 mg/L within 12 minutes. Under the same conditions, there was greater than 2 logs inactivation of HPC bacteria and greater than 5 logs inactivation of *E. coli*. *Giardia muris* was ozonated with the same water and it was found that 1 mg/L ozone residual produced 2.7 logs inactivation within 12 minutes. Finch and Fairbairn (1991) found that 0.040 mg/L ozone residual inactivated more than 4 logs of MS2 coliphage within 20 s in phosphate buffer. Additionally, poliovirus type 3 was paired with the MS2 coliphage and it was found that MS2 coliphage had, on average, 1.6 logs higher inactivation.

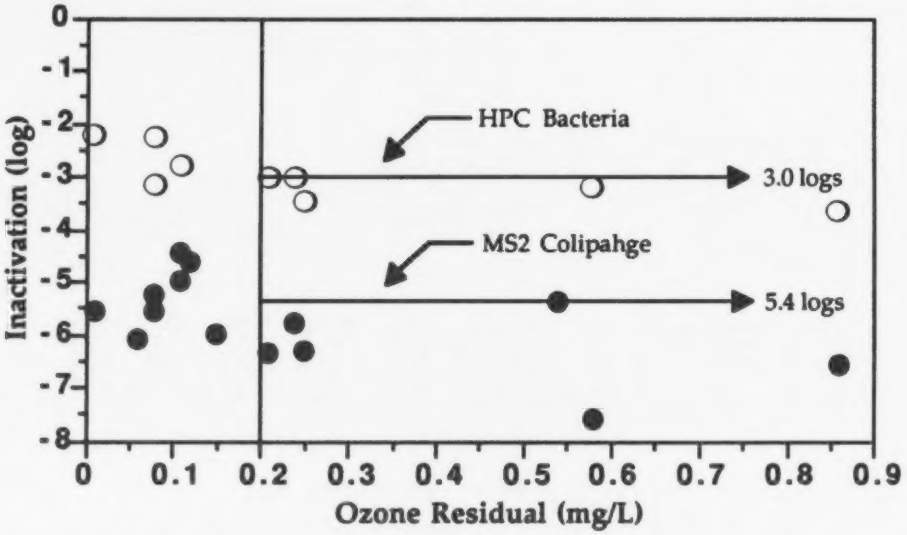


Figure 5.4 - Inactivations of MS2 Coliphage and HPC Bacteria

Finch and Fairbairn (1991) summarized previous reported ozone inactivations of poliovirus (types 1, 2 and 3) in phosphate buffer. In general, most of the reported (Majumdar *et al.*, 1973; Katzenelson *et al.*, 1974; Evison, 1978; Roy *et al.*, 1981; Herbold *et al.*, 1989) inactivations were equal to or less than 4 logs with most inactivations less than 3 logs for ozone residuals spanning from 0.10 to 0.24 mg/L. The reported inactivations of poliovirus were consistently lower than the results of this study and other studies which involved ozone inactivation of coliphages. Any attempt at estimating enteric virus inactivation by using MS2 coliphage would, most likely, overestimate the inactivation of the virus.

The ozone residual was noted (Farooq *et al.*, 1977a) to be the most important factor with inactivation of yeast and acid fast bacteria (*M. fortuitum*). This appears true for HPC bacteria and MS2 coliphage as well. The inactivations shown in Figure 5.4 include test conditions with winter and spring runoff water batches. Many of these data points had high DOC and relatively high applied ozone dose. Once the ozone demand was satisfied and a lasting ozone residual developed, the consistently higher inactivations were observed.

A comparison of ozone inactivation of HPC bacteria and MS2 coliphage of this study with other health related microorganisms (summarized by Sobsey, 1989) is shown in Table 5.1 below.

Table 5.1 - Inactivation of Microorganisms by Ozone

Microorganism	Water Type	Residual (mg/L)	Time (min)	Reduction (%)	Est. Ct mg-min/L
Escherichia coli	?	0.04 - 0.07	0.08 -0.5	99	0.006-0.02
Total Coliforms	Wastewater Effluent	5.0	18	99.89	NR
Fecal Streptococci	Wastewater Effluent	5.0	18	99	NR
Escherichia coli	Wastewater Effluent	0.29 - 0.36	0.6	99.94	NR
Salmonella typhimurium	Wastewater Effluent	0.29 - 0.36	0.6	99.9998	NR
Mycobacterium fortuitum	Wastewater Effluent	0.29 - 0.36	0.6	89	NR
Mycobacterium fortuitum	Demand Free Buffer	0.8 - 1.08	0.58	99	0.53
Poliovirus 1	Demand Free Buffer	0.15 - 0.2	0.4 - 1.5	99	0.2
Poliovirus 2	Demand Free Buffer	0.15	4.83	99	0.72
Poliovirus 1	Wastewater Effluent	0.29 - 0.36	0.6	99.5	NR
Poliovirus 1	Wastewater Effluent	0.2	10 - 15	97	NR
Poliovirus 1	Wastewater Effluent	0.2	10 - 15	80	NR
Rotavirus SA11	Demand Free Buffer	0.1 - 0.3	0.12 - 0.19	99	0.02-0.06
Rotavirus SA11	Wastewater Effluent	0.26	15	96	NR
Human Rotavirus	Wastewater Effluent	0.26	15	40	NR
Human Rotavirus	Wastewater Effluent	0.4	15	99.99	NR
Giardia muris	Demand Free Buffer	0.15 - 0.7	2.8 - 12.9	99	1.94
Giardia lamblia	Demand Free Buffer	0.11 - 0.48	0.94 - 5.0	99	0.53
Naegleria gruberi	Demand Free Buffer	0.55 - 2.0	2.1 - 7.8	99	4.23
Naegleria (6 species)	Distilled Water	0.4	4	>98.9	NR
Acanthamoeba polyphaga	Distilled Water	0.4	4	95	NR
Acanthamoeba (3 species)	Distilled Water	0.4	4	98.9	NR
MS2 Coliphage*	Raw Surface Water	0.20	0.5	99.9	0.01
HPC Bacteria*	Raw Surface Water	0.20	0.5	99.9	0.01

(After Sobsey, 1989) NR = Not Reported * This Study from Bench Scale Results

The estimated Ct values of this study (shown above in Table 5.1) were calculated by multiplying the contact time (i.e. 0.5 minutes) by the ozone residual at the end of the contact time (0.20 mg/L). From the data in Table 5.1, there is widely varying disinfection results depending on the water quality and microorganism. The most resistant microorganisms were the protozoan cysts whereas the least resistant microorganisms were the MS2 coliphage and other viruses. The studies using wastewater appeared to required either a higher ozone dose and/or longer contact time for at least 1 log inactivation. It is difficult to directly compare the inactivation results of this study and those inactivations reported by Sobsey (1989) since the experimental conditions may be considerably different.

5.4 COMPARISON OF MS2 COLIPHAGE AND HPC BACTERIA INACTIVATION

The dose-response curves for MS2 coliphage and HPC bacteria were both similar for low DOC water. A significant similarity of both dose-response curves was the tailing effect ("L" or hockey stick shape) seen at higher applied ozone doses. One possible reason for the tailing is that inactivation kinetics had changed for due to relatively different sensitivities within the MS2 coliphage and HPC bacteria populations. Tailing in a viral dose-response curve may indicate that a very high number of infectious units are small or single unit particles and the few remaining clumps are very large. The survival of the large clumps do not show up until all the smaller units are inactivated (Chang, 1966). For example, a sensitive virus with 99.9 percent single particles and 0.1 percent very large particles would have an immediate 3 logs inactivation while the same virus with 90 percent single particles and 10 percent large clumps would have only 1 log of immediate inactivation. The tailing found in the HPC bacteria may have resulted from the different sensitivities to ozone among the HPC bacteria population (although clumping cannot be ruled out). Some of the HPC bacteria would be particularly sensitive to ozonation creating an initial high rate of inactivation. Other bacteria are more resistant which would form a long tail on a dose response-curve. Additionally, some of the bacteria may have been embedded in organic matter or in clumps further changing disinfection kinetics (Hoff, 1978). Haas (1990) summarized several main reasons for tailing which include:

- Changing of resistances due to inactivation (i.e. hardening)
- Widely varying resistances among each subspecies
- Physical protection of a microorganism
- Variation of chemical dose
- Clumping of the microorganism

In addition to the reasons outlined above, a decrease in the microbial population may have relative changes in the reaction kinetics. Consider the first order rate equation for ozone reacting with a subspecies of microorganisms:

$$\frac{d[O_3]}{dt} = -k\,[M]\,[O_3]$$

Where k is the reaction rate constant of ozone with the subspecies, M. As the ozone inactivation proceeds, the decreasing microorganism population slows the reaction rate which may show up as tailing in the dose-response curve because the contact times were similar in each case.

5.5 PILOT SCALE INACTIVATION

MS2 coliphage was equally as sensitive to ozonation in pilot scale as in bench scale. For any measurable ozone residual, at least 4 logs of MS2 coliphage inactivation was seen. Figure 5.5 shows that the inactivation of MS2 coliphage is identical for either bench or pilot scale experiments. Differences between the inactivation of bench scale and pilot scale experiments would be difficult to determine by the fact that the MS2 coliphage was so sensitive to ozonation.

Ozone bubbles were considered to enhance the inactivation of yeasts and acid fast bacteria (Farooq *et al.*, 1977a). The bubbles would have relatively high ozone gas concentration which, when in contact with the microorganisms, would have instant inactivation. Additionally, the pilot scale reactor would have longer contact time between the dissolved ozone residual and the microorganisms allowing for more opportunity for inactivation. The effect of bubbles or longer contact times in the pilot scale reactor could not be effectively measured because high inactivation of MS2 coliphage was achieved in both pilot and bench scale experiments.

The calculated Ct values were included on Table 4.6 for the various pilot scale inactivation experiments. The AWWA tabulated Ct values (Malcolm Pirnie Inc. and HDR Engineering Inc., 1991) for surface water sources indicate that the required Ct value for 4 logs virus inactivation at 20°C is 0.5 mg/L-min (for ozone). Table 4.6 shows that the lowest Ct value was 0.04 mg/L-min and 5.39 logs MS2 coliphage inactivation was achieved. Moreover, one of the points did not have a residual (i.e. zero Ct) and still had 3.62 logs inactivation. The regulatory Ct basis (Malcolm Pirnie Inc. and HDR Engineering Inc., 1991) is based on more resistant viruses for inactivations. Moreover, MS2 coliphage inactivations would overestimate the inactivation required compared to the published Ct values.

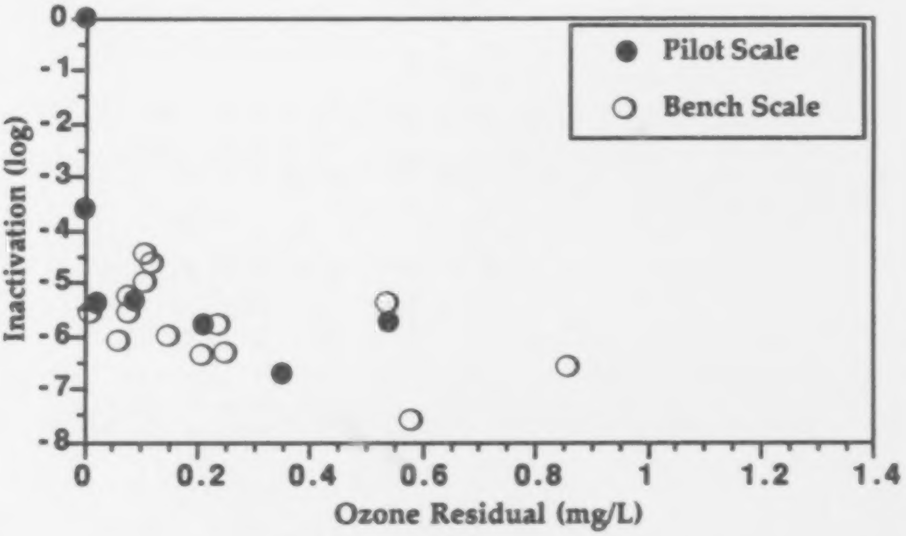


Figure 5.5 - Inactivation of MS2 Coliphage in Bench and Pilot Scale Experiments

Pilot scale HPC bacteria inactivations were conducted and compared to bench scale inactivations in Figure 5.6 using water batch RW3101 for the pilot scale and RW2702 and RW2204 for the bench scale tests. The pilot scale HPC inactivations were considerably lower than the bench scale inactivations for similar ozone residuals. Only up to 1.2 logs inactivation occurred for an ozone residual of 0.09 mg/L, however, between 2 and 3 logs of HPC inactivation occurred in the bench scale experiments. It may be that bench scale inactivation provides better contact between ozone and bacteria than the pilot scale reactor. Additionally, some ozone bubbles may have entered into the sample port falsely suggesting a higher ozone residual than would actually be in the reaction vessel. More pilot scale ozonation experiments using HPC bacteria would be required to determine the actual reason for this anomaly.

The contact time of the pilot scale was considerably higher than the bench scale. The Cts calculated in the HPC pilot scale experiments were fairly low ranging between 0.04 to 0.26 and achieved between 0.4 to 1.2 log inactivation.

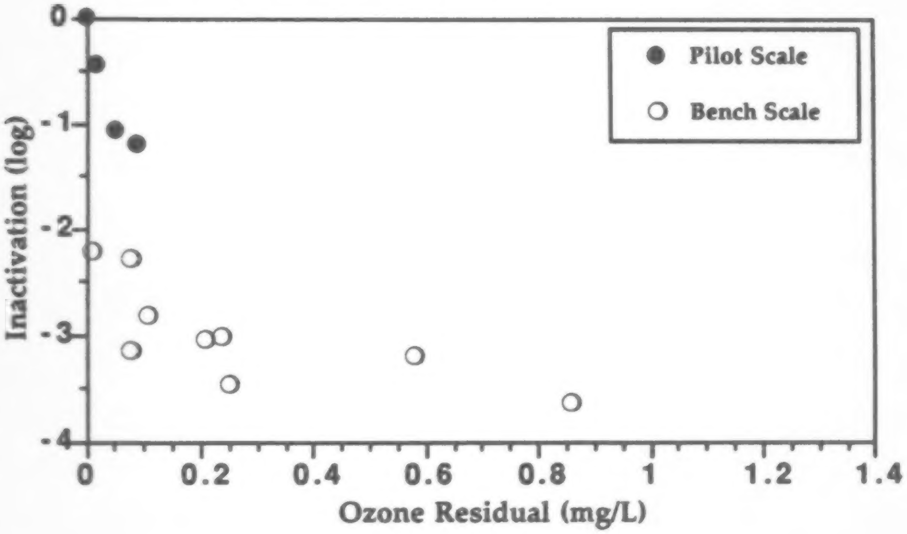


Figure 5.6 - Inactivation of HPC Bacteria for Pilot and Bench Scale Experiments

Two of the pilot scale experiments were conducted with *G. muris* cysts. A comparison of the inactivations of all three pilot scale experiments is shown in Figure 5.7. It appears that the HPC bacteria and the *G. muris* cysts have fairly similar inactivations. The *G. muris* cysts have a lower inactivation level than the MS2 coliphage. The tabulated Ct values (Malcolm Pirnie Inc. and HDR Engineering Inc., 1991) for inactivation of *G. lamblia* cysts suggest that 0.72 mg/L-min is required for 3 logs inactivation at 20°C. The estimated Ct from the *G. muris* inactivation indicated that a Ct of approximately 0.4 to 2.19 mg/L-min would be required. This is fairly similar to the tabulated values relative to Ct requirements of other disinfectants.

One of the main differences between the bench and pilot scale systems is the mass transfer of ozone into solution. In the bench scale system the mass transfer was completed before being added to the reaction vessel. However, in the pilot scale system the ozone was added in the gas form and had to be absorbed into the liquid phase before any residual could be measured. This mass transfer of ozone could have a significant effect on the inactivation kinetics especially in colder water where the mass transfer of ozone may be greater (USEPA, 1986). Furthermore, the hydraulics of the bench and pilot scale reactors are different. The bench scale system has a smooth cylinder shaped magnetic stir bar which rotates at the very bottom of the reactor vessel. The hydraulic flow pattern is generally circular. The pilot scale reactor has a rotating turbine with six flat blades and four baffles inside the reaction vessel which creates a more turbulent flow pattern. The flow pattern of the bench scale reaction vessel may allow "pockets" of higher ozone concentration immediately prior to complete mixing since this vessel did not maximize hydraulic mixing. The flow pattern of the pilot scale reaction vessel would allow a more homogeneous mixing pattern and may achieve a more uniform applied ozone dose to the water.

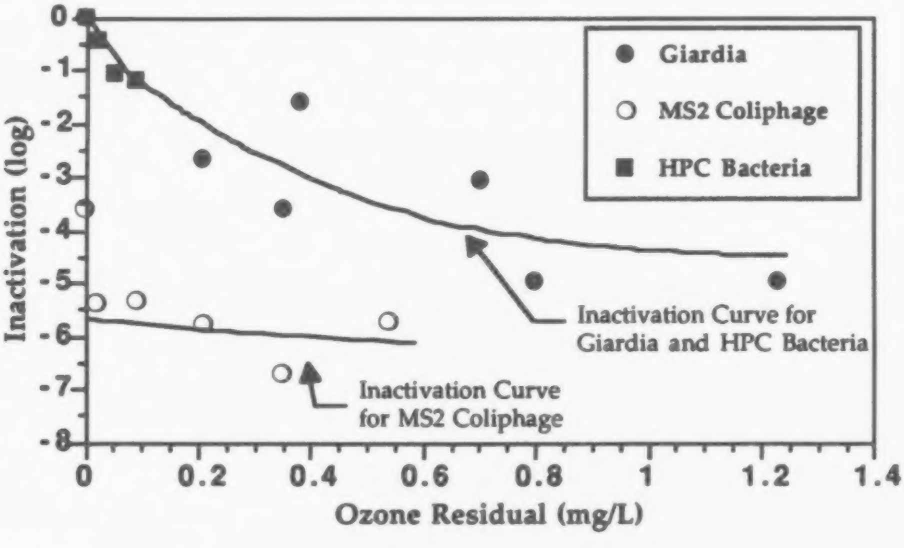


Figure 5.7 - Comparison of Inactivations of *G. muris*, MS2, and HPC in Pilot Scale Experiments

5.6 OZONE DEMAND

The ANOVA results of Table 4.9 indicated that the ozone demand was strongly dependent upon the DOC concentration and the temperature of the water. The higher the DOC concentration, the greater the concentration of compounds that will react with the ozone. Some of the compounds will readily react with ozone in water while other compounds may require higher ozone concentrations or longer contact times.

In terms of DOC concentration, ozone demand had a significant effect on the inactivation of MS2 coliphage. The immediate ozone demand consumed the ozone before the MS2 coliphage could react with the ozone. The immediate ozone demands would have to be satisfied prior to substantial MS2 inactivation thereby requiring a much larger applied ozone dose. Additionally, ozone facility sizing would be directly impacted by the immediate ozone demand during spring runoff. Failure to consider the effect of the immediate ozone demand could result in a facility which is too small and inadequate for disinfection.

It must be noted that the ozone demands may come from a variety of sources and that the ozone demand compounds may also change seasonally. Most of the organics during the winter may not have the same reaction kinetics (i.e. immediate ozone demand) as would the same concentration of organics resulting from spring runoff. Not only should the DOC concentration be considered but also the type of organic material. The literature review indicated that humic material causes color in the water and color immediately disappears upon ozonation. This was also evident during experiments with RW0304. The water was visually noted for having a brownish yellow color which immediately disappeared when ozonated. Furthermore, it was this same water which had a significant ozone demand and relatively low inactivation of MS2 coliphage. It can be concluded that the North Saskatchewan River has seasonal variations which affect the immediate ozone demand of the water which, consequently, effects the inactivation kinetics.

Temperature was important to the ozone demand of the water. Lower water temperatures resulted in lower ozone demands. Temperature may also be important to the inactivation of MS2 coliphage. The higher temperatures had a higher inactivation rate. It appears that ozone may prefer to react with DOC at the lower temperatures (compared to the MS2 coliphage) even though the reactions of DOC with ozone had slowed down considerably. At the higher temperatures, ozone may have reacted preferentially with the MS2 coliphage rather than the DOC since the temperature was found significant. Temperature is only one of the factors important to the inactivation kinetics of ozone. Other factors which affect the ozone reaction rate kinetics are the pH, alkalinity, competing reactions, mass transfer, UV light, ozone concentration, reactant concentrations, etc. Any of these factors may be important to the inactivation of viruses and bacteria. However, when all these factors are in some way involved in the reaction, only the most important factors may show up as significant to ozone inactivation (i.e. applied ozone dose, DOC). For example, the effects of DOC could easily overshadow the effects of pH or turbidity in water where the direct reactions are dominant.

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 SUMMARY

Factorial design experiments were conducted investigating the effects of naturally and seasonally varying parameters on ozone disinfection of MS2 coliphage and HPC bacteria. The parameters studied include pH, total hardness, turbidity, DOC concentration, and temperature. A one-way ANOVA was used to analyze the main effects and interactions of these parameters in conjunction with the applied ozone dose. Most experiments were paired with both MS2 coliphage and HPC bacteria to investigate the relative inactivation difference between MS2 coliphage and HPC bacteria. These results were compared with reported literature to establish the relative differences between the inactivations obtained in this study with those of other studies.

Three pilot scale experiments were conducted with MS2 coliphage, HPC bacteria and *Giardia muris* cysts. The pilot scale MS2 and HPC inactivations were compared to those of the bench scale inactivations. *Giardia muris* inactivations were compared to the pilot scale inactivations of the MS2 coliphage and HPC bacteria.

Ozone demand experiments investigated the effects of organic carbon concentration, temperature, and filtering. Some samples were filtered through a 0.45 µm membrane filter to remove all the suspended organic carbon.

6.2 CONCLUSIONS

Conclusions reached during this study include:

- MS2 coliphage was extremely sensitive to ozonation.
- MS2 coliphage reacted very quickly with ozone.
- DOC had an adverse effect on the inactivation of MS2 coliphage.
- The presence of a detectable ozone residual resulted in greater than 4 logs MS2 inactivation independent of contact time.

- Temperature may have had an effect on ozone inactivation by increasing the inactivation at higher temperatures.
- The effects of pH (between 7.0 and 8.0) and turbidity (between 1.3 and 20 NTU) were not important for inactivation of MS2 coliphage.
- MS2 coliphage was equally sensitive at pilot scale and bench scale.
- MS2 coliphage was more sensitive to ozonation than either of HPC bacteria and *Giardia muris*.
- HPC bacteria were not sensitive to ozonation at the conditions used for MS2 inactivation.
- DOC adversely affected inactivation of HPC bacteria.
- Temperature (between 4 and 22°C), pH (between 7.0 and 8.0), and turbidity (between 1.3 and 20 NTU) were not found to significantly affect ozone inactivation of HPC bacteria.
- The presence of a detectable ozone residual resulted in greater than 2 logs HPC inactivation.
- Immediate ozone demand was affected by the dissolved organic carbon concentration and temperature. The suspended organic carbon did not have any effect on the immediate ozone demand.

6.3 RECOMMENDATIONS

Several recommendations may be made regarding the conclusions brought forward from this investigation. The recommendations include:

- The use of MS2 coliphage as a surrogate for ozone disinfection should be reevaluated. The MS2 coliphage is very sensitive to ozone disinfection and would likely overestimate the capabilities of viral inactivation of ozone.
- HPC bacteria were relatively highly resistant to inactivation. Some of the genera in this group of bacteria may be more suitable for use as an

ozone disinfection indicator. Speciation and population variation over different seasons should be investigated.

- Pilot testing of ozone installations should be done prior to design to determine the ozone dose to achieve a desired Ct. This testing should be conducted in different seasons to determine the effect the spring runoff may have on the applied ozone dose and ozone demand.
- Ozone demand experiments should be conducted at applied ozone dosages which are similar to those which will used in the ozone disinfection experiments. Higher applied ozone dosages may suggest higher immediate ozone demands. Furthermore, applying the ozone dose in smaller doses but longer contact times could be effective in reducing the ozone demand and yet achieve the required disinfection capabilities.

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APPENDIX A - MEAN SQUARE OF ERROR FOR MS2 AND HPC

The mean square of error for the ANOVAs for the MS2 coliphage and HPC bacteria inactivations were calculated as indicated below:

Table A.1 - Mean Square of Error Calculations for MS2 and HPC Inactivations

Experiment	MS2 Kill	Sum of Squares	HPC Kill	Sum of Squares
RW0802	6.10			
	5.51	0.17405		
RW2702 Exp #1	5.15		2.20	
	5.20	0.00125	1.93	0.03645
RW2702 Exp #2			1.56	
			1.42	0.00980
RW2702 Exp #3	4.57		0.37	
	4.16	0.08405	0.33	0.00080
RW2702 Exp #4			0.30	
			0.32	0.00020
RW0304 Exp #1			0.85	
			1.33	0.11520
RW0304 Exp #3	0.77			
	0.73	0.00080		
	0.67			
	0.45	0.02420		
RW0304 Exp #4	1.17			
	1.42	0.03125		
RW2204 Exp #2	5.28		2.30	
	4.99	0.04205	2.82	0.1352
RW2204 Exp #3	6.33		3.48	
	6.39	0.00180	3.06	0.08820
		$\Sigma SS = 0.35945$		$\Sigma SS = 0.38585$
		$\nu = 8$		$\nu = 7$

Therefore the mean square of error for the MS2 coliphage inactivation was calculated as:

$$MS_d = \frac{\Sigma SS}{\nu}$$

$$MS_d = \frac{0.35945}{8}$$

$$MS_d = 0.04493 \text{ with 8 degrees of freedom}$$

And the mean square of error for the HPC bacteria inactivation was calculated as:

$$MS_d = \frac{0.38585}{7}$$

$$MS_d = 0.05512 \text{ with 7 degrees of freedom}$$

APPENDIX B - MEAN SQUARE OF EFFECTS FOR MS2 AND HPC

Analysis of the results was done by one-way analysis of variance (ANOVA) as described by Davies (1979) and in Section 3.3.1. The linear regression coefficient, β, was determined using a linear regression computer program Data Desk Professional. The total effect of a water parameter was found by:

$$TE = 2\beta 2^{m-n-1}$$

Where, β = regression coefficient
m = factorial design order
n = fraction of factorial design
TE = total effect

The mean square of the effect was found by:

$$MS = \frac{TE^2}{v2^{m-n}}$$

Where, v = degrees of freedom (typically 1)
MS = mean square of effect

Significant effects and interactions were determined by dividing the mean square of effect by the mean square of error and comparing it to tabulated (Box *et al.*, 1978) F-statistic values.

Any mean square of effect greater than 5 percent significance level ($Pr \leq 0.05$) was considered significant while any effect greater than 1 percent significance level was considered highly significant.

Table A.2 and A.3, below, shows the regression coefficients for each parameter along with the calculated total effect and mean square for the MS2 coliphage and HPC bacteria, respectively.

Table A.2 - Regression Coefficients, Total Effect, and Mean Square for MS2

Experiment	Parameter	β	Total Effect	Mean Square
RW0802	Ozone Dose	0.1925	0.7700	0.148225
	pH	-0.0575	-0.2300	0.013225
	Ozone Dose-pH	-0.0425	-0.1700	0.007225
RW2702 Exp #1	Turbidity	0.165	0.6600	0.1089
	pH	0.075	0.0225	0.0225
	Turbidity-pH	-0.090	-0.0324	0.0324
RW2702 Exp #3	Ozone Dose	2.315	9.260	21.4369
	pH	-0.070	-0.280	0.0196
	Ozone Dose-pH	0.090	0.360	0.0324
RW2702 Exp #4	Ozone Dose	2.405	9.620	23.1361
	Turbidity	0.230	0.920	0.2116
	Dose-Turb	0.240	0.960	0.2304
RW0304 Exp #3	Ozone Dose	0.8605	3.442	2.96240
	DOC	-0.9782	-3.913	3.82731
	Dose-DOC	-0.6414	-2.566	1.64571
RW0304 Exp #4	Ozone Dose	1.03125	8.25	8.50781
	DOC	-0.85625	-6.85	5.86531
	Temperature	0.08875	0.71	0.06301
	Dose-DOC	-0.77875	-6.23	4.85161
	Dose-Temp	0.17125	1.37	0.23461
	DOC-Temp	0.04875	0.39	0.01901
	Dose-DOC-Temp	-0.04875	-0.39	0.01901
RW2204 Exp #2	DOC	0.080	0.32	0.0256
	Temperature	0.410	1.64	0.6724
	DOC-Temp	0.200	0.40	0.1600
RW2204 Exp #3	DOC	-0.54	-2.16	1.1664
	Temperature	0.04	0.20	0.0064
	DOC-Temp	-0.33	-0.33	0.4356

Table A.3 - Regression Coefficients, Total Effect, and Mean Square for HPC

Experiment	Factor	β	Total Effect	Mean Square
RW2702 Exp #1	Turbidity	0.035	0.140	0.0049
	pH	-0.200	-0.800	0.1600
	Turbidity-pH	0.005	0.020	0.0001
RW2702 Exp #2	Turbidity	-0.0325	-0.130	0.004225
	Total Hardness	-0.2875	-1.150	0.330625
	Hardness-Turb	0.0325	0.130	0.004225
RW2702 Exp #3	Ozone Dose	1.050	4.20	4.4100
	pH	-0.185	-0.740	0.1369
	Ozone Dose-pH	-0.210	-0.840	0.1764
RW2702 Exp #4	Ozone Dose	0.095	0.190	0.01805
	Turbidity	0.010	0.020	0.00020
	Dose-Turbidity	-	-	-
RW0304 Exp #1	Ozone Dose	0.1275	0.510	0.065025
	DOC	-0.2225	-0.890	0.198025
	Dose-DOC	-0.1275	-0.510	0.065025
RW0304 Exp #3	Ozone Dose	0.245	0.980	0.2401
	DOC	-0.260	-1.040	0.2704
	Dose-DOC	-0.095	-0.380	0.0361
RW2204 Exp #2	DOC	0.465	0.930	0.43245
	Temperature	0.220	0.440	0.09680
	DOC-Temp	-	-	-
RW2204 Exp #3	DOC	-0.120	-0.480	0.0576
	Temperature	-0.120	-0.480	0.0576
	DOC-Temp	0.100	0.400	0.0400

APPENDIX C - MEAN SQUARE OF ERROR FOR OZONE DEMAND TESTS

The mean square of error calculated for the ozone demand experiments were calculated from the data listed below in Table A.4.

Table A.4 - Mean Square of Error for Ozone Demand Tests

Experiment	Ozone Demand (mg/L)	Sum of Squares (SS)	Degrees of Freedom (v)
RW1801	1.24		
	1.41	0.01445	1
RW2501	1.03		
	0.58	0.10125	1
RW3101	1.21		
	1.35	0.00980	1
RW2702	0.64		
	0.61	0.00045	1
RW0304	2.40		
	2.17	0.02645	1
	2.30		
	2.27	0.00045	1
	3.55		
	3.53	0.00020	1
Milli-Q Water	-0.15		
	0.00		
	0.01		
	-0.32		
	-0.04	0.08300	4
RW2204	1.84		
	1.92	0.00320	1
	1.13		
	1.13	0.00000	1
		$\Sigma SS = 0.23925$	$\Sigma v = 13$

The mean square of error is then calculated as:

$$MS_d = \frac{0.23925}{13}$$

$MS_d = 0.01840$ with 13 degrees of freedom

APPENDIX D - MEAN DIFFERENCE BETWEEN MS2 AND HPC INACTIVATIONS

The mean difference between all the paired MS2 coliphage and HPC bacteria inactivations were calculated below in Table A.5.

Table A.5 - Paired Results for MS2 and HPC Inactivations

Experiment	MS2 Inactivation (log)	HPC Inactivation (log)	Difference (log)
RW1302	3.62	0.00	3.62
	5.39	0.45	4.94
	5.36	1.08	4.28
RW2702 Exp #1	5.07	2.17	2.90
	5.40	1.76	3.64
	5.58	2.23	3.35
	5.55	1.84	3.71
	5.15	1.93	3.22
	5.20	2.20	3.00
RW2702 Exp #3	0.83	0.12	0.71
	0.51	0.17	0.34
	5.28	2.64	2.64
	5.32	1.85	3.47
	4.57	0.37	4.20
	4.16	0.33	3.83
RW2702 Exp #4	0.29	1.06	-0.77
	4.64	1.23	3.41
	5.58	1.25	4.33
	2.49	0.32	2.17
RW0304 Exp #3	0.78	0.41	0.37
	0.15	0.08	0.07
	3.59	1.09	2.50
	0.67	0.38	0.29
RW2204 Exp #2	5.80	3.02	2.78
	5.14	2.53	2.61
	6.36	2.09	4.27
	4.99	2.82	2.17
	5.28	2.30	2.98
RW2204 Exp #3	6.59	3.65	2.94
	7.33	3.21	4.12
	6.17	3.21	2.96
	5.59	3.17	2.42
	6.39	3.06	3.33
	6.33	3.48	2.85
	4.44 ± 2.04	1.69 ± 1.15	2.75 ± 1.39

The t-test for matched pairs may be calculated by:

$$t = \frac{\overline{D} - \Delta}{s_D/\sqrt{n}}$$

Where,

$$s_D = \sqrt{\frac{\Sigma(D_i - D)^2}{n - 1}}$$

From the above data, we have $s_D = 1.39$ with 34 sample points and also D (bar) = 2.75. The null hypothesis is Ho: no significant difference exists between the MS2 and HPC inactivations, and the alternative hypothesis is Ha: a significant difference exists between the MS2 and HPC inactivations. The calculated t statistic is:

$$t = \frac{2.75 - 0}{1.39 / \sqrt{34}}$$

$$t_{calc} = 11.6$$

The tabulated (Box *et al.*, 1978) t statistic for 95% significance level, two sided and 30 degrees of freedom is 2.021. Therefor, the calculated t statistic is significantly greater than the tabulated indicating that the mean difference is significant.

APPENDIX E - SUMMARY OF INACTIVATION EXPERIMENTS

Table A.6 - Summary of MS2, HPC, and *Giardia* Inactivations

Exp.	Appl. Ozone mg/L	Resid. Ozone mg/L	MS2 Kill log	HPC Kill log	*Giardia* Kill log	DOC mg/L	Turb. NTU	pH	Temp. °C	Total Hard. meq/L	Cont. Time min
1411		0.38			1.6	7.04	6.0	8.23	22	3.52	3.17
		0.70			3.1	7.04	6.0	8.23	22	3.52	6.25
1801	0.73	0.34	3.01			1.21	2.9	8.15	22	3.58	0.33
	1.12	0.56	4.52			1.21	2.9	8.15	22	3.58	0.33
	0.36	0.00	3.50			1.21	2.9	8.15	22	3.58	0.33
	0.78	0.21	3.71			1.21	2.9	8.15	22	3.58	0.33
	0.17	0.00	1.71			1.21	2.9	8.15	22	3.58	0.33
2501	0.04	0.00	0.22			2.04	2.4	8.19	22	3.65	0.33
	0.20	0.00	3.09			2.04	2.4	8.19	22	3.65	0.33
	0.44	0.00	2.97			2.04	2.4	8.19	22	3.65	0.33
	0.58	0.17	5.69			2.04	2.4	8.19	22	3.65	0.33
	0.82	0.19	6.04			2.04	2.4	8.19	22	3.65	0.33
3101	0.00	0.00	0.00			1.62	3.0	8.30	22	3.52	0.00
	1.47	0.21	5.79		2.7	1.62	3.0	8.30	22	3.52	4.0
	2.44	0.35	6.74		3.6	1.62	3.0	8.30	22	3.52	6.0
	2.93	0.54	5.77			1.62	3.0	8.30	22	3.52	8.0
	3.67	0.80			5.0	1.62	3.0	8.30	22	3.52	10.0
	4.95	1.23			5.0	1.62	3.0	8.30	22	3.52	13.5
0802	0.37	0.14	6.00			1.66	3.2	7.83	22	3.42	0.33
	0.39	0.15	6.20			1.66	3.2	7.05	22	3.42	0.33
	0.19	0.00	4.98			1.66	3.2	8.05	22	3.42	0.33
	0.20	0.00	5.73			1.66	3.2	7.07	22	3.42	0.33
	0.30	0.00	5.51			1.66	3.2	7.43	22	3.42	0.33
	0.29	0.06	6.10			1.66	3.2	7.43	22	3.42	0.33
1302	0.00	0.00	0.00	0.00		1.61	2.6	8.10	11	3.20	0.00
	0.14	0.00	3.62	0.00		1.61	2.6	8.10	11	3.20	1.83
	0.28	0.02	5.39	0.45		1.61	2.6	8.10	11	3.20	3.58
	0.34	0.05		1.08		1.61	2.6	8.10	11	3.20	4.35
	0.45	0.09	5.36	1.21		1.61	2.6	8.10	11	3.20	5.67

Table A.7 - Summary of MS2, HPC, and *Giardia* Inactivations

Exp.	Appl. Ozone mg/L	Resid. Ozone mg/L	MS2 Kill log	HPC Kill log	*Giardia* Kill log	DOC mg/L	Turb. NTU	pH	Temp. °C	Total Hard. meq/L	Cont. Time min
2702	0.22	0.00	5.55	1.84		1.20	13.0	7.98	22	3.20	0.33
Exp #1	0.22	0.00	5.58	2.23		1.20	1.5	7.96	22	3.20	0.33
	0.21	0.01	5.15	1.93		1.20	6.7	7.52	22	3.20	0.33
	0.22	0.00	5.40	1.76		1.20	14.0	7.04	22	3.20	0.33
	0.22	0.00	5.20	2.20		1.20	6.8	7.49	22	3.20	0.33
	0.22	0.00	5.07	2.17		1.20	1.3	7.04	22	3.20	0.33
Exp #2	0.21	0.00		1.23		1.20	1.3	8.01	22	9.19	0.50
	0.22	0.00		1.56		1.20	7.3	8.05	22	6.25	0.50
	0.22	0.00		1.42		1.20	7.2	8.05	22	6.18	0.50
	0.21	0.00		1.87		1.20	1.3	7.99	22	3.19	0.50
	0.22	0.00		1.23		1.20	20	8.03	22	9.06	0.50
	0.21	0.00		1.74		1.20	19	7.99	22	3.19	0.50
Exp #3	0.22	0.00	5.32	1.85		1.20	1.5	8.04	22	3.20	0.50
	0.04	0.00	0.51	0.17		1.20	1.5	8.04	22	3.20	0.50
	0.23	0.00	5.28	2.64		1.20	1.5	7.01	22	3.20	0.50
	0.05	0.00	0.83	0.12		1.20	1.5	7.01	22	3.20	0.50
	0.14	0.00	4.57	0.37		1.20	1.5	7.53	22	3.20	0.50
	0.13	0.00	4.16	0.33		1.20	1.5	7.53	22	3.20	0.50
	0.00	0.00	0.01			1.20	1.5	7.96	22	3.20	0.00
Exp #4	0.05	0.00	0.29	1.06		1.20	14.0	8.00	22	3.20	0.50
	0.23	0.00	4.64	1.23		1.20	2.5	8.04	22	3.20	0.50
	0.05	0.00	0.31			1.20	2.5	8.04	22	3.20	0.50
	0.13	0.00		0.30		1.20	6.4	8.02	22	3.20	0.50
	0.13	0.00	2.49	0.32		1.20	6.4	8.02	22	3.20	0.50
	0.22	0.00	5.58	1.25		1.20	14.0	8.00	22	3.20	0.50
	0.00	0.00	-0.03			1.20	2.5	7.96	22	3.20	0.00

Table A.8 - Summary of MS2, HPC, and *Giardia* Inactivations

Exp.	Appl. Ozone mg/L	Resid. Ozone mg/L	MS2 Kill log	HPC Kill log	*Giardia* Kill log	DOC mg/L	Turb. NTU	pH	Temp. °C	Total Hard. meq/L	Cont. Time min
0304	0.13	0.00		0.85		4.73	18.0	7.98	22	2.83	0.50
Exp #1	0.14	0.00		1.33		4.73	18.0	7.98	22	2.83	0.50
	0.23	0.00		0.70		3.93	14.0	8.03	22	2.83	0.50
	0.04	0.00		0.19		3.93	14.0	8.03	22	2.83	0.50
	0.22	0.00		0.00		6.68	25.0	8.01	22	2.83	0.50
	0.05	0.00		0.00		6.68	25.0	8.01	22	2.83	0.50
Exp #3	0.31	0.00	0.77			3.33	6.2	7.95	22	2.83	0.50
	0.45	0.00	0.67	0.38		5.20	9.1	8.02	22	2.83	0.50
	0.23	0.00	0.15	0.08		5.20	9.1	8.02	22	2.83	0.50
	0.22	0.00	0.78	0.41		1.92	3.2	8.07	22	2.83	0.50
	0.46	0.00	3.59	1.09		1.92	3.2	8.07	22	2.83	0.50
	0.34	0.00	0.73			3.33	6.2	7.95	22	2.83	0.50
	0.40	0.00	0.45			5.20	9.0	8.05	22	2.83	0.50
Exp #4	0.22	0.00	0.55			4.85	39.0	7.78	22	2.83	0.50
	0.22	0.00	0.51			1.50	15.0	7.58	22	2.83	0.50
	0.44	0.00	0.78			4.85	38.0	7.69	4	2.83	0.50
	0.33	0.00	1.17			2.91	27.0	7.64	22	2.83	0.50
	0.22	0.00	0.87			1.50	15.0	7.56	4	2.83	0.50
	0.00	0.00	0.01			6.09	48.0	7.83	22	2.83	0.50
	0.43	0.00	4.05			1.50	15.0	7.31	4	2.83	0.50
	0.19	0.00	0.52			4.85	40.0	7.74	4	2.83	0.50
	0.42	0.00	1.30			4.85	38.0	7.62	22	2.83	0.50
	0.44	0.00	4.57			1.50	14.0	7.58	22	2.83	0.50
	0.33	0.00	1.42			2.91	26.0	7.62	22	2.83	0.50

Table A.9 - Summary of MS2, HPC, and *Giardia* Inactivations

Exp.	Appl. Ozone mg/L	Resid. Ozone mg/L	MS2 Kill log	HPC Kill log	*Giardia* Kill log	DOC mg/L	Turb. NTU	pH	Temp. °C	Total Hard. meq/L	Cont. Time min
2204	0.84	0.00	6.36	2.09		2.96	9.3	7.96	22	3.42	0.50
Exp #2	0.86	0.11	4.99	2.82		2.22	7.1	7.98	22	3.42	0.50
	0.85	0.24	5.80	3.02		2.16	4.7	7.81	22	3.42	0.50
	0.86	0.08	5.28	2.30		2.22	6.9	8.01	22	3.42	0.50
	0.86	0.00	5.14	2.53		2.96	9.5	7.98	4	3.42	0.50
	0.91	0.54	5.38			2.16	4.8	7.83	4	3.42	0.50
Exp #3	1.42	0.21	6.39	3.06		2.10	14.0	8.05	22	3.42	0.50
	1.42	0.08	5.59	3.17		2.88	20.0	8.12	22	3.42	0.50
	1.47	0.86	6.59	3.65		1.34	7.8	7.92	4	3.42	0.50
	1.45	0.25	6.33	3.48		2.10	14.0	8.03	22	3.42	0.50
	1.42	0.00	6.17	3.21		2.88	19.0	8.03	4	3.42	0.50
	1.55	0.58	7.61	3.21		1.34	8.5	7.89	22	3.42	0.50

APPENDIX F - SUMMARY OF OZONE DEMAND RESULTS

Table A.10 - Summary of Ozone Demand Results

Experiment	Applied Ozone (mg/L)	Residual Ozone (mg/L)	Ozone Demand (mg/L)	Temperature (°C)	DOC (mg/L)	Filtered (Y/N)
RW1801	2.745	1.496	1.249	22	1.210	No
	3.922	2.510	1.412	22	1.210	No
RW2501	3.643	2.615	1.028	22	2.040	No
	3.292	2.712	0.575	22	2.040	No
RW3101			1.210	22	1.616	No
			1.350	22	1.616	No
RW0802	0.380	0.141	0.239	22	1.664	No
	0.744	0.462	0.282	22	1.664	No
RW2702	0.085	0.043	0.042	22	1.197	No
	0.168	0.079	0.088	22	1.197	No
	0.246	0.102	0.144	22	1.197	No
	0.319	0.153	0.166	22	1.197	No
	0.201	0.058	0.142	22	1.197	No
	0.207	0.095	0.112	22	1.197	No
	0.201	0.080	0.121	22	1.197	No
	4.003	3.360	0.643	22	1.197	No
	3.786	3.176	0.610	22	1.197	No
RW0304	4.853	2.397	2.456	22	4.731	No
	4.997	2.171	2.826	22	4.731	No
	4.797	2.497	2.300	22	3.930	No
	4.877	2.611	2.266	22	3.930	No
	5.084	1.531	3.553	22	6.680	No
	4.934	1.400	3.534	22	6.680	No
Milli-Q	3.301	3.453	-0.152	22		
	3.347	3.349	-0.002	22		
	3.338	3.332	0.007	22		
	3.356	3.671	-0.315	22		
	3.271	3.320	-0.049	22		
RW2204 Exp #1	3.194	2.920	0.274	4	1.340	Yes
	3.288	2.640	0.648	22	1.340	Yes
	3.295	2.937	0.358	4	1.500	No
	3.704	3.257	0.447	22	1.500	No
	3.800	2.600	1.200	22	2.882	Yes
	3.534	2.914	0.620	4	2.903	No
	3.217	2.485	0.732	4	2.882	Yes
	3.421	2.223	1.198	22	2.903	No
RW2204 Exp #2	3.704	3.671	0.033	4	1.619	Yes
	3.854	3.624	0.230	22	1.619	Yes
	3.651	3.680	-0.029	4	1.619	No
	3.708	3.294	0.414	22	1.619	No
	3.511	2.876	0.635	4	2.962	Yes
	3.483	2.437	1.046	22	2.962	Yes
	3.561	3.062	0.499	4	2.985	No
	3.612	2.576	1.036	22	2.985	No